"IMMUNOLOGICAL PROFILE IN NEW BORN BABIES".

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C E R T I F I C A T E

This is to certify that the work entitled "IMMUNOLOGICAL PROFILE IN NEWBORN BABIES", which is being submitted as Thesis for M.D.(Paediatrics) examination 1992 of Bundelkhand University, Jhansi, has been carried out by Dr. RAJEEV SHARMA himself in this department.

He has put in the necessary stay in the department as required by the regulations of Bundelkhand University.

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C E R T I F I C A T E

This is to certify that the present study entitled, "IMMUNOLOGICAL PROFILE IN NEWBORN BABIES" has been carried out by Dr. RAJEEV SHARMA, under my direct supervision and guidance. All the findings have been checked and verified by me from time to time. The technique was actually undertaken by the candidate himself.

He has also fulfilled all the conditions necessary for the submission of thesis.

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This is to certify that Dr. Rajeev Sharma has worked on "IMMUNOLOGICAL PROFILE IN NEWBORN BABIES" under my guidance and supervision. His results and observation have been checked and verified by me from time to time.

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Dated: 27th Oct. 1991 (RAJEEV SHARMA)

C O N T E N T S

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INTRODUCTION

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Infections in newborn still remain a major problem despite advances in chemotherapeutic agents. The long held concept of the neonate as an "immunologically null" subject is no longer appropriate. A corner stone of the present century lies in the enormous advances made by umpteen workers in the field of immunology. Strange though it may seem, not many immunologists have delved to study the immunological status of the newborn, specially in certain neonatal disorders known to affect their immunological profile. The study thus throws challenge to most pioneer workers in the field, not only in unearthing the intricate mechanisms of neonatal defence, but also in evaluating the extent to which the neonate can combat the challenges of environment, after a relatively secure place in the mother's womb. Although neonatal period is the most significant period in the human life yet not much work has been done so far to know the effect of various disorders over the immunological status of the newborn. The present study has been aimed towards this need and is a small effort to unveil the challenge faced by immunologists.

With the advancement of immunology, one could find immunological explanation for every disease and further diagnosis of the disease by implication of the same. There is a paucity of quantitative data on the levels of immunoglobulins and components of complement in serum of the healthy newborn babies and variation in their status in different diseases. Neonatal period is the most vulnerable period in the human life to acquire various infections. What is the effect of various diseases on neonates and their immunological status ? Why all neonates do not respond in a similar fashion to the same disease ? These few questions still remain an enigma.

Materno-foetal immunologic relationship is perhaps unique in biological world. We see that mother provides all essential immunoglobulins to the newborn, to survive till it starts producing its own immunoglobulins, in the form of passive immunity which it acquires transplacently and through colostrum.

Studies done so far, have shown that there is a state of diminished immune responsiveness of a normal healthy newborn baby, with subsequent impaired resistance to various antigens and micro-organisms in the extra-uterine environment. The decreased state of immunological function is seen to be related to the depressed specific and non-specific immune mechanisms in newborns. During

intra-uterine period, fetus generally lacks antigenic challenge but has the ability to respond, however there are maturation deficiencies in complement activity, immunoglobulin content and defective phagocytic response leading to diminished inflammatory response in the newborn.

Besides uncompromised non-specific immune status in this age, certain factors like low birth weight, which may be accounted by prematurity or intra-uterine malnutrition or both, may further adversely affect the developing immune apparatus of the newborn. Fetal malnutrition is also seen to affect the post-natal immune competence by hampering the development of the thymic dependent areas which are responsible for cellular immunity. The depressed state of immune responsiveness in these newborns thereby pre-disposes them to various infections which in turn may alter their immunological profile and this immunological response may determine the course of the disease.

Although unconjugated bilirubin has long been known to have toxic effect on various body tissues specially the brain cells, only meagre attention has been drawn on its effect on the immune apparatus of the newborn. Studies done in the recent past have unravelled a depressed state of immune responsiveness of the newborn infant following neonatal hyperbilirubinemia. The awareness

that this depression may be long lasting prompted the authors in highlighting the importance of an early therapeutic intervention and subsequent follow-up.

In India there are more chances of exposure to infections of a neonate. Also the health of the mother and environment is responsible for the various neonatal problems in the newborn. To know the effects of various infections on the neonates and their response in relation to these stimuli, it is essential to assess the immunological status of the newborn.

The state of the art concerning the complement system is tenuous. Although much progress has been made in the past 15 years, an extra-ordinary amount is still unknown about the basic immuno-chemistry of this system and its relationship to human disease, specially in the neonates. Research in this area is cumbersome with many inexact and semi-quantitative methods. To our knowledge, no formal study of the activity as C_3 complement of complement system in the serum of neonates with hyper-bilirubinemia, small for gestational age and neonates having infection has been reported. Therefore, in view of the possibility of increased infection rate in these groups of neonates, this aspect deserves attention.

The immune state of a neonate forms the baseline of any study of immune response in man, as active immune

responses become operative immediately after exposure to the antigenic stimuli from environment. In this country, these stimuli become operative quite early, as an average neonate has a greater chance of an exposure to infection fairly early in life and hence it is mandatory to know the immune status of the newborn to decrease the morbidity and mortality, by finding the high risk newborns and by providing them special care.

Present work is directed at studying mainly the humoral immune response in the normal newborn babies and those suffering from some common neonatal disorders.

Following are the aims of the present study :

1. To assess humoral and complement activity of normal full term healthy newborn babies.

2. To assess humoral and complement activity in low birth weight babies, which includes both appropriate for gestational age premature babies and small for date babies suffering from intra-uterine malnutrition.

3. To assess humoral and complement activity in cases of neonatal hyperbilirubinemia.

4. To assess humoral and complement activity in cases of neonatal infections.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

All human beings are susceptible to infections irrespective of their age, sex, race. However, few are more exposed to infection than others, especially newborns. The immunoglobulins are a family of proteins which may be identified in the plasma or serum by immunochemical methods. The main components have been identified (Franklin, 1962), viz. IgG, IgA, IgM, IgD and IgE.

Infants depend primarily upon placentally transferred immunity for protection of infection in early life. Data collected subsequently revealed that, cellular immunity also plays a significant role in host defense. Both humoral and cellular immune mechanisms impact resistance to traumatic experiences just after birth.

General Immune Response -

Immunogen entering the body excites two types of reactions (i) initial non-specific immune reaction (NSIR) comprising an inflammatory response with subsequent phagocytosis, and (ii) specific immune reaction (SIR) comprising antibody dependent and cell mediated reactions.

Antigen - Host - - Non-specific immune reaction (NSIR)
- Specific immune reaction (SIR).

The NSIR is mediated by phagocytic cells including those of mononuclear phagocytic system (MPS), polymorphonuclear leucocytes (PMN), eosinophils and mediator cells including mast cells, basophils and platelets.

The specific immune reaction as a whole is dependent upon the nature of the immunogen/antigen. The site of response is lymphnodes and spleen and the mediators include T and B lymphocytes, killer cells, natural killer cells and macrophages. The complement and Kallikrein systems assist the antibody dependent reactions mediated by B-cells.

The chief function of lymphocyte is generation of immunity by a complex phenomenon, culminating in the synthesis of specific immunoglobulin (antibody) and establishment of cellular immunity.

The antibody activity of serum and other body secretions is associated with a heterogenous group of proteins, collectively known as immunoglobulins (Ig). These proteins are also known as gamma globulins because of their relative electrophoretic mobility. Many antibodies migrate more rapidly than gamma globulins and some molecules unrelated to antibodies, may also migrate with the electrophoretic mobility of gamma globulins. For these reasons

the term "Immunoglobulins" and symbol "Ig" or " " has been suggested to designate the family of molecules with antibody activity (Committee of Nomenclature of Human Immunoglobulin - Bull. WHO, 1964, Fahey, 1965).

Gamma globulins, by virtue of their antibody activity, play a significant role in resistance of infection. However, antibody alone may not be sufficient to resist the infection since the ultimate destination or localization of invading organisms depends upon the interaction of antibody and defence mechanism. Antibody potentiates the migration of bacteria by phagocytes (Samuel et al, 1970).

During health, fairly stable levels of immunoglobulins are maintained in the plasma due to the state of equilibrium between the rate of synthesis and catabolism. Levels are subject to vary due to wide spectrum of antigenic stimuli. The variations would also be expected due to difference in environmental, racial and genetic factors and socio-economic status.

In a developing country like India, where a relatively higher incidence of subclinical infection is likely to be encountered, the healthy population would reveal some diversity in the levels of immunoglobulins of normal population as already established in Western literature.

There are three main types of immunoglobulins with antibody activity, which are immunoglobulin G (IgG),

Immunoglobulin M (IgM), and Immunoglobulin A (IgA). Recently, two more proteins with immuno-chemical characteristics related to these immunoglobulins, Immunoglobulin D (IgD) and Immunoglobulin E (IgE) have been detected.

Despite the tremendous heterogenicity, all the immunoglobulins share structural similarity. All consist of a basic sub-unit composed of four polypeptide chains, held together by disulphide bonds.

According to Eldaman and Poulik (1961) gamma globulins could be split into two components by mild reduction with thiols in the presence of urea. Each of the two components consisted of polypeptides - 1. The heavy chain (molecular weight 50,000) and 2. light chain (molecular weight 20,000). Authors further observed that about 75% of an IgG molecule was made up of heavy chain while the remaining 25% consisted of light chains.

Kunkel and Grey (1964) discovered the sub-class of IgG, viz. (IgG_1, G_2, G_3 and G_4).

Rowe and Fahey (1965) discovered the fourth class of immunoglobulin viz. IgD from an atypical myeloma proteins which did not react with any of the known anti IgG anti IgA or anti IgM sera. Kunkel and Prendergast (1966) discovered the sub-class of IgA, while Ishizaka and Hornbook (1966)

discovered the fifth class of immunoglobulins IgE during their study of reaginic antibodies.

Hong et al (1972) classified the immunoglobulins in five major classes (IgG, IgM, IgA, IgD and IgE) on the basis of their general property, structural differences of their heavy chains including the amino acid sequence and length of the polypeptide chain. He summarized the chemical, biological and metabolic characteristics of immunoglobulins which are included in the table given.

Immunoglobulin G (IgG) -

IgG is the major immunoglobulin and constitutes about 3/4th of total gamma-globulin. IgG has a sedimentation constant of 7S and contains about 90% of acquired antibodies. It has been observed that the concentration of gammaglobulin in the plasma of newborn infant is equal to or greater than that of their mothers because of selective transfer across the placenta. After birth the level is minimum between 3-6 months of age. The concentration then rises again to reach adult levels between first and second year of life. Synthesis of immunoglobulin G occurs at 11th week of gestation (Cocchi et al, 1969 and McCraken et al, 1971). It has a molecular weight of 150,000 daltons. IgG is the only immunoglobulin that crosses the placenta and thereby provides maternal antibodies to neonate (Fahey, 1965). Mean serum concentration of IgG in cord blood of a newborn baby is usually in the range of 740-1650 mg% (Hardy et al,1969)

TABLE

Biological and metabolic characteristics of Immunoglobulins modified from Byung, H. Park, Robert, A., Good (1974) and Hong et al (1972).

	IgG	IgM	IgA		IgD	IgE
			Serum	Secretory		
Molecular weight	140,000	90,000	160,000	370,000	160,000	197,000
Half life (days)	25 - 35	9 - 11	6 - 8		2 - 3	1 - 2
Production (mg/kg/day)	28 - 36	5 - 8	8 - 10		0.4	-
S. concentration (mg/100 ml)	800 - 1600	50 - 200	60 - 420		3	-
Transplacental passage	+	-	-		-	-
Complement fixation	+	++	-		-	-
Secreted by mucous surface	±	±	±	+	?	+
Polymer formation	-	+	+	+	-	-
Blocking antigen	+	-	-	+	-	-

which consists mostly of maternal antibodies and falls to a level of about 200 - 600 mg% by about six months of age. As the infant is exposed to antigenic environment IgG levels gradually start increasing reaching adult levels (i.e. 800 - 1200 mg%) by about four years of age.

On the basis of antigenic determinants within the heavy chain of IgG, four isotypic classes of IgG molecules have been identified in the normal serum. These are IgG_1 (66%), IgG_2 (23%), IgG_3 (7%), IgG_4 (4%). Following are the mean serum IgG levels in cord blood as given by different authors : 740 - 1650 mg% (Hardy et al, 1969), 348 - 2000 mg% (Evans et al, 1971), 612 - 654 mg% (Malik et al, 1977), 860.68 - 1312.13 mg% (Sethi et al, 1980), 1402 $\pm$ 132.3 mg% (Sharma et al, 1986) and 1120 - 1692 mg% (Kolhatkar et al, 1987).

Immunoglobulin A (IgA) -

IgA is the next most abundant immunoglobulin in serum. IgA globulin has a sedimentation constant between 69 and 135. It has been shown to be absent from the plasma of newborn infant as it does not cross the placenta and its synthesis begins at about the 2nd to the 4th week of life. Not much is known about the antibody content of this fraction but recent studies shows that some of the skin sensitizing antibodies in certain allergic individuals and Brucella abortus and diphtheria antibodies may belong to

this fraction. The class IgA comprises about 10 percent of the gamma globulin in human serum. IgA class can be sub-divided into two separate systems of Immunoglobulins. One of these provides IgA antibodies for internal secretions synthesized by non-mucosal lymphoid tissue. The other system of IgA antibodies are found in external secretions. IgA of external secretions in most parts is not derived from blood but is produced locally by plasma cells. IgA synthesis is virtually undetectable in the fetus and do not become substantial for several months after birth. Serum levels of IgA is 0 - 3.9 mg% (Malik et al, 1977) at birth and gradually increases to about 25 - 75 mg% by two years of age. Adult levels 200 to 300 mg% are reached in adolescence (Tomasi et al, 1968 and Soloman et al, 1973). Secretory IgA have antibody activity against a variety of viruses, bacteria (Clancy and Bienestock, 1976). Molecular weight of serum IgA is 160,000 daltons and of secretory IgA is 370,000 daltons. Following are the levels of serum IgA in cord blood as estimated by different workers : 0 - 46 mg% (Evans et al, 1971), 0 - 30.9 mg% (Malik et al, 1977); 9.12 $\pm$ 10.04 mg% (Kaur et al, 1979); 25.4 $\pm$ 5 mg% (Hariharan et al, 1984).

Immunoglobulin M (IgM) -

IgM is the largest of the polymeric immunoglobulins, usually being a pentamer of the H_2 L_2 structure with one J chain. IgM immunoglobulin has a sedimentation constant of

19S, this fraction, like IgA globulin is incapable of crossing the placenta. But its synthesis can occur at a slow rate in the foetus. After birth, the rate of synthesis increases rapidly and it has been reported that adult level may be reached by the 9th month of age. Recent work has shown that the newborn infants are by no means immunologically incompetent as it was once thought to be. Molecular weight of IgM is 900,000 daltons. IgM is also known as macro-globulin due to its high molecular weight. IgM is produced in the primary response to antigenic challenge. IgM is the main immunoglobulin produced by the fetus and while the amount formed is usually small when there is fetal infection, substantial IgM response may occur. Serum concentration of IgM in a newborn is about 1.6 to 31 mg% (Hardy et al, 1969) which rapidly increases to adult level of 50 - 150 mg% by about 1 year of age. The IgM level in serum is between 5 and 10 percent of the total antibody protein. Following are the cord serum levels of IgM as reported by different workers - 1.6 to 31 mg% (Hardy et al, 1969), 0 - 25 mg% (Khan et al, 1974), 0 - 20.8 mg% (Malik et al, 1977), 9.12 $\pm$ 10.04 mg% (Kaur et al, 1979), 22.8 - 84.4 mg% (Hariharan et al, 1984) and 12.1 $\pm$ 13.5 mg% (Sharma et al, 1986).

Immunoglobulin D (IgD) -

IgD is found in very low concentration in the serum, 0.3 to 40 mg% (Rowe and Fahey, 1965). IgD was

discovered by Rowe and Fahey having a molecular weight of 180,000 daltons and found mainly in the intravascular space and on resting B cells as a cell surface immunoglobulin. IgD comprises about 3 mg percent of normal serum immunoglobulins.

Immunoglobulin E (IgE) -

IgE is also known as reagenic antibody, mediates acute, sometime life threatening allergic reactions in atopic patients. It has a molecular weight of 190,000 daltons and binds to basophils and mast cells when a specific antigen combines with the antigen binding site on IgE. The serum concentration of IgE is in the range of 0.01 to 0.07 mg% with a mean of 0.03 mg% (Bennich et al, 1971).

Human Complement C_3 -

This complement component is present in human serum in a concentration of approximately 1.2 mg/ml which is by far the largest amount of any complement in serum (Lundh, 1964; West et al, 1964; Klemperer et al, 1965; Kohler and Muller-Eberhard, 1967). Its sedimentation coefficient is 9.5 'S' and the molecular weight is estimated to be approximately 240,000. It is readily demonstrated by immuno-electrophoresis of whole human serum, the corresponding precipitin being located in the B-globulin region and partially within the transferrin arc (Muller-Eberhard et al, 1960).

Complement plays an integral role in host defence against infection. The deficiency of complement components in neonatal serum has been implicated to be cause of defective opsonic activity and chemotactic activity (Forman et al, 1969). The synthesis of C_3 complement takes place in fetal liver (Miller, M.E., 1971) and no transplacental transfer has been documented (Miller, 1978). Out of all the complement components C_3 is present in highest concentration in adult and neonatal serum. Following are the values of mean serum complement C_3 in cord blood as given by various authors : 124.72 $\pm$ 44.62 mg% (Kaur et al, 1979), 90 $\pm$ 18 mg% (Shapiro et al, 1981), 51.4 $\pm$ 14.94 mg% (Tandon et al, 1984).

Immunoglobulins in Neonates -

Susceptibility of the newborns to various infections has been known for a long time. This is particularly the case in premature and small for date infants where infection leads to increased rate of mortality and morbidity. Maternally transmitted immunoglobulin IgG is the main stay of the humoral immunity in postnatal period. Antibodies produced by B-cells are located in the globulin fraction of the serum (Tisellus and Kabat, 1939) and are called immunoglobulins.

Immunoglobulins in the serum can be measured by many methods. Single radial immuno-diffusion method has been found to be simple, easily performed and in wide use especially due to its easy availability in the market

Lymphocytes with IgM, IgG, IgA surface receptors have been demonstrated by immunofluorescence in the peripheral blood, liver spleen, and bone marrow at 11½ weeks of gestation. Normally only IgG can cross the placenta. The presence of IgM and IgA in the cord and newborn sera is the result of active synthesis by the fetus or because of maternal bleeding into the fetal circulation.

Thus we see that a normal full term healthy infant at birth has an incompletely developed immunological system that is why, is more prone to develop infections. Both prenatal and postnatal infections alter the immunological status of the newborns. The immunological status of the newborn is further compromised in low birth weight infants. Hyperbilirubinemia of newborn also plays an adverse effect on the immunological status of the newborn. Following is a brief review of the literature regarding the immunological profile in various neonatal disorders and normal infants.

Immunological profile or Normal healthy full term babies -

Babies with birth weight of more than 2.5 kg, more than 37 weeks of gestational age, without any infection born as a result of normal vaginal delivery have been kept in this group.

IgG -

Synthesis of Immunoglobulin IgG occurs at 11 weeks of gestation (Cocchi et al, 1969 and McCraken et al, 1971). Mainly IgG lies in the maternal blood and is selectively transported through placenta to the fetus. This occurs mainly in III trimester, hence infants born before 34 weeks of gestation have deficiency of IgG. Rate of transfer of IgG depends upon maternal IgG levels as well as the age and function of placenta (Chandra, 1975). A small amount of IgG is however, synthesised in utero by the fetus (Gotoff, 1974). Allansmith et al (1968) have reported higher cord blood IgG levels than maternal blood. These findings were confirmed by Chandra et al (1970). A linear relationship between IgG levels and gestational age was suggested by Evans et al (1971). Raghvan et al (1976) also showed a correlation between the levels of IgG and the gestational age of the neonate. Malik et al (1977) reported higher IgG levels in the full term neonate than the corresponding mother. Similar findings were obtained by Mahambare et al (1978), they also supported the fact that the levels of IgG were directly proportional to the gestational age and not to the birth weight. The levels of serum IgG were directly correlated with the birth weight, and period of gestation (Kaur et al, 1979). Sethi et al (1980) showed a linear correlation of serum IgG levels with gestational age. In a study conducted by Hariharan et al (1984), it was concluded that the IgG levels were high at birth and in agreement with values

reported by Western and Indian workers. No correlation was seen between maternal and cord serum IgG levels, the cord serum IgG levels were significantly correlated with gestational age (Tandon et al, 1985). Sharma et al (1986) reported IgG level in cord blood to be 1402.31 ± 132.31 mg/ 100 ml, which is in agreement with various workers. Kolhatkar et al (1987) reported that serum IgG levels of Indian infants were appreciably higher than their Western counterparts at all ages.

IgM -

The immunoglobulin IgM can be produced by the fetus by 10½ weeks of gestation (Gotoff, 1974) but the levels remains very low at birth (Steihm et al, 1966). Allansmith et al (1968) reported that level of IgM remains constant for the first 5 days after birth, and then increases rapidly for 2 days. Newborn develop 50% level of the adult values of IgM by the end of four months of age and adult levels are attained by the age of 1 to 2 years (Allansmith et al, 1968). Some of the newborns were having high levels than normal infants which was attributed to maternal bleeding into fetal circulation (Sever et al, 1969). Levels of IgM are not related to sex and infants having lower levels of IgM are at a risk of death (Hardy et al, 1969). IgM levels can be determined by a simple radial diffusion method (Khan et al, 1969). Prasad et al,

(1971) observed lower levels of IgM as compared to the reported figures. Evans et al (1971) reported similar levels of IgM among multiple-birth newborns corresponding to those of single infant of same gestational age. Raghvan et al (1976) reported a higher level of IgM in the Indian population as compared to the reports from the west. IgM levels were low and had no relation with the gestational age and birth weight indicating absence of placental transfer and negligible synthesis by the fetus (Mahambare et al, 1978). Higher serum IgM levels in cord blood as compared to Western world, were reported by Hariharan et al,(1984). Sharma et al (1986) reported no significant relationship of cord blood IgM with birth weight and gestational age. A high value of serum IgM was observed in the post-mature babies by Goel et al (1987). Low serum IgM values at birth were reported by Kolhatkar et al (1987) and they increase as the age advances.

Complement C_3 -

Not much work has been published regarding the level of complement in the newborn babies. Subnormal complement activity in cord blood was reported in 1927 by Larrier et al. In 1964, Lundh et al reported that complement C_3 was present in neonates in a concentration of 1.2 mg/ml. Similar findings were confirmed by Kohler and Muller-Eberhard (1967). They also reported that C_3

is the fraction of complement system present in largest amount in the blood. All components except C_3 increased in concentration during the first four days of life (Ballow et al, 1974). In man, synthesis of complement can occur as early as the eighth week of gestation and precedes the appearence of immunoglobulins (Colten et al, 1974). Drew and Arroyave (1978) found a statistically significant correlation between increasing birth weight or gestational age and increasing serum concentration of C_3 fraction.

Complement synthesis begins early in autogeny and preceds immunoglobulin synthesis. It starts at 8th week of intra-uterine life and is established by 11 - 14 weeks. Complement do not cross placenta and normal values of C_3 were ranging between 60 - 200 mg% (Kaur et al, 1979). Shapiro et al (1981) reported values of C_3 in cord blood of healthy neonates of about 90 mg%. When the birth weight was controlled the correlation between the cord serum C_3 levels and gestation ($P \not< 0.05$) became insignificant. Similarly, when gestational age was controlled the correlation between the birth weight and cord serum C_3 levels became insignificant (Tandon et al, 1984). Singh et al (1986) reported that break down product of C_3 were not detected in healthy neonates.

Immunological profile of low birth weight babies -

Babies with birth weight of less than 2.5 kg irrespective of the period of gestation, born by normal vaginal delivery, have been included in this group. The group includes premature babies having weight appropriate for gestational age, as well as small for date newborns. Small for date babies are those who weigh less than expected for the gestational age, the weight falling below the 10th percentile for the period of gestation or 2 standard deviation below the mean weight.

Naeye (1966), described 2 groups of small for date babies on the basis of pathological observations. The first group is of malnourished small for date babies, occurring as a result of foetal malnutrition during the later part of gestation. These infants show diminished amount of cytoplasm in the cells. The second group also called hypoplastic group, is attributed to intra-uterine infections and genetic and chromosomal disorders. This group contains normal amount of cytoplasm in the cells but cells are reduced in number.

IgG -

Hobbs and Davis (1967) measured levels of Immunoglobulins in a group of small for dates (prematures) in the first week of life and observed that there is a linear relation between the level of IgG and gestational age.

All prematures born before 32 weeks gestation had IgG less than 400 mg%.

Yeung and Hobbs (1968) observed that there was a significant decrease in serum IgG levels in both small for date and premature babies as compared to full term infants. They observed a mean serum IgG levels in small for date infants (40, 38½ and 37 weeks of gestation) to be 626, 578 and 512 mg% respectively, as compared to values of 1100, 879 and 757 mg% observed in control group of cases of similar gestational age group. The serum IgG levels in AGA group of infants showed a linear correlationship with increasing gestational age. The lower levels of IgG can be due to the placental insufficiency.

Rothberg (1969) observed that serum IgG levels have got a definite linear relationship with the increase in the weight of the premature infants.

Evans et al (1971) observed that serum IgG levels showed a definite increase with the increase in weight of the premature infants. With decreasing gestational age the median values of IgG declined. At 40 wks, 35 wks, 31 wks and 27 wks the IgG values were 1088, 850, 595 and 430 mg% respectively.

Hyvarinen et al (1973) did not observe a significant decrease in the levels of serum IgG in small for date infants, only 2 of the 8 studied showing a decreased levels of this class of immunoglobulin.

In 1975, Chandra studied the immunological status in 26 normal full term and 26 S.F.D. infants and observed that the serum IgG levels have got a linear relationship with the gestational age. The follow-up of 10 S.F.D. babies showed that not only serum IgG levels were low but the subsequent drop in the IgG levels was also much severe in S.F.D. as compared to the normal full term infants.

Raghvan et al (1976) studied immunoglobulin IgG in sixteen healthy premature neonates and compared the pattern obtained in fifteen healthy full term neonates. They reported IgG values of 170.8 $\pm$ 99.1 I.U./ml in prematures which were significantly lower when compared to the values of 253.6 $\pm$ 137.6 I.U./ml obtained in full term ($P < 0.025$). The levels of IgG in cord sera of premature neonates showed a direct correlation with the birth weight ($P < 0.01$).

Pre-term babies showed significantly low levels of IgG, in a study conducted by Meharban Singh (1978). The neonates with severe IUGR and pre-term babies had significantly lower levels of IgG ($P < 0.01$). The values of immunoglobulin IgG in IUGR were 92.05 $\pm$ 17.03 I.U./ml and in preterms were 86.49 $\pm$ 30.30 as compared to the normal neonates in whom the values were 138.91 $\pm$ 34.75. The pre-term babies had significantly low levels of IgG in the cord blood because materno-fetal transfer of immunoglobulins occurs during third trimester of pregnancy.

Hobbs and Davis (1967) gave a direct relationship between the cord blood IgG and gestational age, similar results were obtained in the study by Singh et al (1978).

Kaur et al (1979) reported in her study that the levels of IgG were directly correlated with the birth weight and period of gestation. Serum IgG levels in pre-term, S.F.D. and controls were 829.66, 1142.06 and 1478.5 mg% respectively. It was observed that the values obtained in both premature and SFD babies were statistically significant from those observed in the control group of babies ($P < 0.001$). This was in agreement with the results concluded by Chandra et al (1976). Raghvan et al (1976) and Mahambare et al (1978). Sethi et al (1980) in a study of 20 L.B.W. newborns (14 SFD and 6 AGA), observed a significant decrease in serum IgG levels of both SFD and AGA infants as compared to control ($P < 0.001$). This decrease was more pronounced in S.F.D. having prematurity as well. The findings also showed a linear correlation between IgG levels and gestational age. The mean serum IgG levels of six premature SFD infants (478.2 mg%) was much less as compared to that of premature AGA infants of same gestational age (687.35 mg%) indicating placental pathology commonly observed in S.F.D. infants.

Shapiro et al (1981) however in their study reported values contrary to those observed by other workers in the field. They studied 28 term newborns of

whom 17 were SGA and 11 were A.G.A. They observed that there was no significant difference between the serum IgG values in both the group (P $\overline{/}$0.005) values being 1363 and 1461 mg% in A.G.A. and S.G.A. respectively.

Tandon et al (1984) reported that the cord serum IgG levels were significantly lower in pre-term babies (712 $\pm$ 207.2 mg%) compared to full term AGA (1450 $\pm$ 478.6 mg%) and full term - IUGR babies (1586 $\pm$ 538.1 mg%). The cord serum IgG levels were significantly correlated with gestational age.

Khatua et al (1984) conducted a study consisting of 20 control, 18 term SFD and 12 premature infants and observed IgG levels of 1040.75 $\pm$ 146.56 mg%, 888.05 $\pm$ 270.4 mg% and 728.38 $\pm$ 120.12 mg% respectively. On statistical evaluation the workers observed a significant difference in the IgG values of premature and S.F.D. as compared to the control group of cases (P $\angle$0.001 and P $\angle$0.05).

Sharma et al conducted a study in 1986 and observed that the mean IgG levels in controls, prematures and F.T. IUGR were 1402.31 $\pm$ 132.31, 1267.3 $\pm$ 84.0, and 1422.0 $\pm$ 102.0. The mean IgG concentration in pre-terms was significantly (P $\angle$0.001) lower than full term newborns.

In a study of S.F.D. done by Bhatia et al (1987), significantly lower cord serum IgG levels were found in

premature babies irrespective of their intra-uterine growth status.

Goel et al (1987) conducted a comparative study of immunological status in pre-term, term and post-term infants and observed the mean IgG level in the three groups was 821.43, 1179.45 and 1328.54 mg% respectively. The difference in the values between term and pre-term group was significant ($P < 0.01$).

IgM -

Yeung and Hobbs (1968) in a study of small for date and AGA infants observed raised IgM levels in 12 out of 28 small for date babies. As increase in the serum IgM level in newborns is known to be the result of intra-uterine infection, the authors suggested that intra-uterine infection might have led to the fetal malnutrition, or alternatively abnormal placentae of the S.F.D. babies may have permitted the entry of organisms in the fetus.

Rothberg (1969) observed in his study that serum IgM levels were independent of the weight and gestational age of the newborn. Hardy et al (1969) observed no significant difference in the serum IgM levels in relation to the weight and sex of the newborn babies.

Evans et al (1971) in an immunological study of premature infants observed that IgM was not detected in cord sera of 37-75% premature infants with standard plates. However, 64 infants showed IgM values 15 mg per 100 ml or more with Low Level Test plates with a range of 1.4 to 27.0 mg per 100 ml. IgM levels did not show any correlation with the length of gestation or birth weight.

Prasad et al (1971) also conducted a study on immunoglobulin levels in twenty four prematures, twenty full term neonates, fifteen infected infants and found that level of IgM in cord blood in premature babies was in the range of 2.8 to 11.6 mg% with a mean of 6.66 $\pm$ 3.6 mg%.

Chandra et al (1975) studied the serum IgM in 26 normal and 26 S.F.D. infants and observed findings similar to other workers i.e. serum IgM levels were having no relationship to birth weight and gestational age.

Raghvan et al (1976) studied serum IgM in sixteen healthy premature and fifteen full term neonates and observed no statistically significant difference in the IgM values of prematures and full terms. Though IgM was detected in all the infants except one premature. Mean level of IgM in the cord blood in prematures was 10.04 $\pm$ 7.51 mg% with a range of 0 to 24.47 mg%.

Mahambare et al (1978) performed a study on cord blood in 50 cases (forty four babies were full term and 6 were prematures) and found that IgM concentration in the cord is not affected either by the gestational age or the birth weight. No significant difference between the two weight groups of full term babies ($t = 0.943$, P = not significant) was observed. In premature babies also cord IgM levels were very low and no difference in the levels with different gestational age was seen.

Singh et al (1978) assessed the immunoglobulin IgM, IgG and IgA in 20 F.T. and 12 prematures and 24 IUGR newborns. They observed no significant difference in the value of cord IgM in the different groups. Cord serum IgM values observed in IUGR, premature and controls were 20.00 ± 19.35, 14.75 ± 14.65 and 18.71 ± 12.75 I.U./ml respectively.

Kaur et al (1979) conducted a study of pre-term S.F.D. and F.T. babies and assessment of humoral immunity was done by single raidal diffusion technique. They observed that IgM was present in the cord blood of only some of the babies in all the three categories at birth that is in six pre-term (40%), two S.F.D. (13%) and ten controls (50%). Cord IgM level was 5.13, 1.76 and 9.12 mg% respectively. Further, they reported that IgM levels are not dependent on the birth weight of the newborn babies.

Shapiro et al (1981) conducted a study of 28 term newborns, of whom 17 were SGA and 11 AGA. They found cord IgM levels of less than 20 mg/ml and observed no significant difference ($P > 0.05$) between the serum IgM levels of the two groups. Placental histology did not reveal any placental infection in SGA patients.

Sharma et al (1986) studied the cord blood of 100 newborns by single radial immuno-diffusion technique and observed that mean serum IgM levels of 11.58 ± 12.58 mg/100 ml. IgM did not show any significant correlation with birth weight and gestational age. The levels of IgM in pre-term, borderline pre-term and full terms were 7.4 ± 2.9, 8.2 ± 5.2 and 12.1 ± 13.5 mg/100 ml respectively.

Bhatia et al (1987) in a study of hundred twelve live born singleton babies observed that serum IgM levels were not having significant difference in the various groups viz. LBW babies, F.T. A.G.A. babies and F.T. IUGR babies.

Goel et al (1987) estimated serum IgM and other immunoglobulins by simple radial immuno-diffusion technique developed by Mancini et al (1965), in the cord blood of 72 normal newborns. Out of these 72 cases there were 28 premature, 32 full term and 12 post-mature neonates. IgM was observed in only 25% of the total cases in which post-mature infants had maximum percentage (66.66%).

The mean serum IgM values in these three sub-groups were 39.40, 44.25 and 45.71 mg% respectively as compared to 118.25 mg% in 12 adult specimens showing significant relationship ($P < 0.01$).

Complement C_3 -

Ballow et al (1974) reported that all components except C_3 increased in concentration compared to maternal levels during the first four days of life. Pre-term infants had less whole complement activity and lower complement concentrations than full term infants. Drew and Arroyave (1978) found a statistically significant correlation between increasing birth weight or gestational age and increasing serum concentrations of complement specially C_3. Concentrations of C_3 have been 60% to 100% of adult concentrations in term infants and some what less in pre-term infants. Younger gestational age has been correlated with lower levels of complement C_3.

Jagadeesan and Reddy (1978) studied levels of serum complement in 54 newborns out of which 25 were S.F.D. The mean serum complement levels in infants weighing more than 2500 gm were 29.4 U/ml. It is observed that there was no significant difference in the complement levels between the S.F.D. group with different birth weights. The serum values in S.F.D. having weight between 2000 gm to 2500 gms were in the range of 25.58 to 29.6 units/ml.

In this study complement activity did not seem to be altered in S.F.D. infants.

Kaur et al (1979) studied the complement activity in pre-term, S.F.D. and term babies and observed that the levels of complement C_3 were lower in the pre-term and S.F.D. babies as compared to that observed in term babies at birth. They also observed that pre-term babies have lower levels of complement in proportion to their immaturity. They opined that complement components do not cross the placenta and further communicated that preterm infants have a defective opsonic activity to all organism or antigens whereas the term newborn serum apparently has decreased opsonic activity only to certain organisms, primarily gram negative organisms.

Shapiro et al (1981) studied 28 term newborns of whom 17 were SGA and 11 AGA and reported that mean C_3 concentration in SGA group was significantly lower ($P < 0.02$) than in the AGA group. C_3 concentration in AGA and SGA infants was 90 mg% and 75 mg% respectively.

Tandon et al (1984) reported that cord serum C_3 levels of 50 low birth weight babies was significantly lower in pre-term when compared to term AGA and term IUGR babies. However, C_3 level was not significantly different between term AGA and term IUGR babies. No correlation could be seen between C_3 levels and birth weight.

The mean levels of complement C_3 in term AGA, pre-terms and term IUGR was 51.5 ± 14.94, 33.8 ± 11.18 and 47.5 ± 19.75 respectively. The cord C_3 level was found to be significantly correlated with the gestational age (Fireman et al, 1969; Steihm et al, 1975; Jagadeesan et al, 1978).

Bhatia et al (1987) reported significantly lower cord serum C_3 levels in pre-term babies irrespective of their intra-uterine growth status. C_3 level was also reduced in F.T. IUGR low birth weight babies when compared to F.T. AGA controls. Both gestational age and birth weight was found to be independent of the complement status.

IMMUNOLOGICAL PROFILE IN HYPERBILIRUBINEMIA CASES

Although neonatal jaundice is a common condition and effect of unconjugated bilirubin on various body tissues is well documented yet not many studies have been done in the past, to observe the correlation of hyperbilirubinemia on the humoral immune status of the neonates. The results of these studies point towards a depressed state of immune responsiveness of the newborn having hyperbilirubinemia.

IgG -

Nejelda (1967) observed the immune status of newborn babies with erythroblastosis fetalis and reported that the levels of serum gammaglobulins as a whole were

significantly decreased in these babies as compared to the healthy neonates. The suppression of antibody response was thought to be due to the impairment in the development of the cells involved in immune system, and because of the toxic effect of hyperbilirubinemia on the reticulo-endothelial system.

Ansaldi et al (1968) conducted a study to find out the effect of hyperbilirubinemia on the humoral immune response and reported no significant change in the levels of immunoglobulins IgG in neonates with bilirubin levels above 16 mg% when compared to normal healthy controls.

Nejelda (1970) in another study done later reported that high serum bilirubin levels suppressed the immune response in newborns during the 1st week of life as well as in those infants who were followed up for 1 year.

Mantalenaki et al (1975) evaluated the effect of exchange transfusion on the serum immunoglobulin levels in the neonates with hyperbilirubinemia and found variable effects on different immunoglobulins. Exchange transfusion had a prolonged inhibitory effect on the synthesis of serum IgG and IgA. The exact mechanism of this phenomenon was not clear but the levels of all classes of immunoglobulins were same in infants with hyperbilirubinemia not having received exchange transfusion and in controls.

Several explanations have been given to explain the suppression of the immune response with increasing concentration of bilirubin. Nejelda (1970) and Pleszezynski et al (1975) put forward the hypothesis of the toxic effects of hyperbilirubinemia on the viability of the immunological status of the cells. Pleszezynski et al (1975) on the experimental basis observed that hyperbilirubinemia interfers on same metabolic pathways in the production of immunity viz. damage of the lymphocyte membrane by bilirubin. Hirshchorn and Hirshchorn (1965) and Noir et al (1972) suggested that the detrimental effect on immunity may be due to interaction of increasing bilirubin levels with the lysosomal membrane or due to inhibition of function of mitochondria respectively.

Sethi et al (1989) reported no significant alteration in serum IgG levels as compared to control. Thirty cases of neonatal hyperbilirubinemia were studied. The serum values of IgG in control and hyperbilirubinemia group were 1102.58 $\pm$ 134.43 and 995.87 $\pm$ 163 mg% respectively. P value was >0.05.

IgM -

Ansaldi and associates (1968) evaluated the effect of bilirubin on humoral immunity and reported slightly lower levels of IgM in neonates with bilirubin levels above 16 mg% when compared to normal healthy infants not having jaundice.

Complement C_3 -

So far there is no study reported in the literature about the effect of bilirubin on the complement system and their components specially C_3.

IMMUNOLOGICAL PROFILE IN CASES OF NEONATAL INFECTIONS

The immune status of a neonate forms the baseline of any study of immune response in man as active immune responses become operative immediately after exposure to the antigenic stimuli from the environment. In this country, these stimuli became operative quite early as an average neonate has a greater chance of an exposure to infection fairly early in life. The low level of immunoglobulins specially IgM at birth makes the neonate more susceptible to gram negative infections.

IgG -

Alford et al (1967) in a study of infected neonates observed no change in the level of serum IgG in the control and the neonatal infection group.

McCraken et al (1969) studied 2600 serum samples. Serum IgG was studied in 88 cord sera with congenital rubella and compared with the values in controls. No significant difference was observed in the values of serum IgG in the control and the study groups.

Similar observations were recorded by Sever (1969) and Chandra et al (1970) in a study of the effects of neonatal infections on the immunological profile.

Prasad et al (1971) studied twenty normal neonates and 15 neonates with acute infections alongwith prematures and observed that there was no significant difference in the mean serum IgG levels in the control and infected neonates, the values of IgG were 444.6 $\pm$ 20.4 mg% and 479.8 $\pm$ 32.4 mg% respectively.

Malik et al (1977) in a study of immunoglobulins in the neonates born of mothers suffering from infection during their antenatal period, premature rupture of membrane, toxaemia of pregnancy and malformed full term neonates reported that serum IgG levels were raised in the premature rupture of membrane group as compared to control group, and possibly it was due to the infection in mother leading to increase in the serum IgG levels of the neonates due to passive placentral transfer. No significant difference was seen between the serum IgG levels in the controls and the baby born to infected mothers, the values were 253.6 $\pm$ 137.5 and 243.6 $\pm$ 107.3 mg% respectively.

Mahambare et al (1978) reported that the mean 8th day serum IgG level in the neonates was slightly lower than the cord level of IgG in the controls. Though it was

not significant (t = 1.1, P not significant), it may indicate that the infant had yet out begun to synthesis their own IgG.

Mehta et al (1987) studied immunological profile in 70 septicemic and 40 normal neonates to evaluate its usefulness as a diagnostic and prognostic tool in neonatal septicemia. Serum IgG levels were significantly lower in septicemic neonates. Decreased IgG levels in serum correlated with a poor outcome among septic newborns.

IgM -

Alford et al (1967) evaluated from their study of acutely infected neonates postnatally, that high levels of IgM beginning from the third day after onset of infection.

Sheldon et al (1969) in another study of 57 cases suffering from septicemia observed that serum IgM levels were raised in all the cases, the rise was 1st to appear in newborns with pneumonia.

John L. Sever (1969) reported that IgM immuno-globulin levels are often elevated in infants in association with congenital and perinatal infections.

Hardy et al (1969) reported an increase in the levels of serum IgM in newborns having septicemia as well as in those whose mothers had suffered from respiratory infections during pregnancy.

Prasad et al (1971) observed an appreciable rise in the serum IgM fractions in response to infection. They studied twenty normal and fifteen neonates with acute infections and observed serum IgM values in controls and infection group to be 17.88 ± 1.77 mg% and 39.2 ± 8.36 mg% respectively.

Blankenship et al (1974) also observed an increase of IgM in 80% of cases and reported a greater increase in viral as compared to the bacterial infections. Further they reported that staphylococci were most antigenic among various bacteria; they studied.

Malik et al (1977) studied the immunological profile of neonates exposed to the risk of perinatal infections. They found that the levels of serum IgM were significantly raised in newborns born to mothers having definite history of acute infection, during pregnancy. The levels of IgM were normal in neonates born after premature rupture of membranes. The failure of IgM levels to increase in the neonates born after premature rupture of membranes was explained by the authors to be due to the failure of the infective stimulus to reach the fetal immune system. The values of IgM in control and infected group newborns were 11.38 ± 6.76 and 98.7 ± 58.7 mg% respectively.

Khatua et al (1984) studied humoral immunity, morbidity and mortality from infective diseases of 50 newborns and reported that cord serum IgM values were significantly raised ($\nearrow$ 20 mg%) in infants whose mother had infective ailments during pregnancy.

Mehta et al (1987) studied serum IgM levels in 70 septicemic and 40 normal neonates to evaluate its usefulness as a diagnostic and prognostic tool in neonatal septicemia. The workers, reported significantly higher serum IgM levels in cases of septicemia than in their control group of cases.

Complement C_3 -

Johnston et al (1979) expressed their views regarding the role of complement in the host defence mechanism. Though complement plays an integral role in the host defence against infection but with the possible exception of viruses (Mills and Cooper, 1978), complement does not appear to play an important role in resistance to infection by intra-cellular parasites. Whether the newborn infant is actually predisposed to infection because of the complement deficiencies, remains to be proved.

Tandon et al (1984) studied serum C_3 levels in infected newborns and concluded that the low cord serum C_3 levels predispose neonates to increased risk of

infection due to (i) lower opsonic activity as low C_3 levels cause lesser enhancement of IgG and IgM activity, and (ii) deficient chemotactic activity.

Singh (1986) studied thirty two neonates with clinical and bacteriological evidence of infection. Twenty four healthy neonates served as controls for their study. Blood samples were taken for complement estimation. The worker reported that the infected neonates showed breakdown products of complement component C_3 and these breakdown products were detected in 34.4% of infected patients. However, breakdown products of C_3 were not detected in any of the healthy controls. The worker concluded from the above study that complement breakdown products of C_3 can be utilized as a diagnostic tool in case of neonatal infections.

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MATERIAL AND METHODS

MATERIAL AND METHODS

This prospective study was conducted in the Department of Pediatrics, M.L.B. Medical College and Allied Hospital, Jhansi, in active collaboration with the Department of Obstetrics and Gynaecology, over a period of one year from May 1990 to April 1991. The cases included in the study, were selected from the newborns delivered in the hospital and those admitted in the Pediatric ward of the M.L.B. Medical College and Allied Hospital, Jhansi. All newborn babies were broadly divided into four sub-groups for assessment of their immunological profile.

1. Normal full term healthy newborn babies (Control).
2. Low birth weight infants -
   (a) Appropriate for gestational age (AGA) premature infants.
   (b) Small for date (SFD) babies which included mature as well as premature babies.
3. Infants with neonatal hyperbilirubinemia.
4. Infants with neonatal infections.

SELECTION OF CASES

Cases were selected in different sub-groups according to the following selection criteria.

Control : Twenty full term healthy newborns served as control for the present study. Care was taken to exclude all those factors which could adversely affect the immunological status of these newborns. The criteria of selection of these cases was -

1. Weight above 2500 gm.
2. Gestational age ranging from 37 to 41 weeks.
3. Apgar score at the time of delivery varying from 7 to 10.
4. There was no history of infection, toxaemia, diabetes, prolonged rupture of membranes in the mother during pregnancy and labour.
5. None of the newborns were suffering from any infection or congenital malformation.

Blood samples were taken from the umblical cord in all the control cases at the time of birth.

Low birth-weight babies :

Twenty low birth weight newborns were taken for the present study. These included ten appropriate for

gestational age premature babies, and ten small for date (S.F.D.) infants, having weight less than 10th percentile for gestational age. The gestational age was assessed by the date of last menstrual period, and by physical characteristics given by Usher's criteria (1965). The criteria for selection of these cases was -

1. All the premature babies had gestational age below 37 weeks.

2. The S.F.D. babies were those showing features of intra-uterine malnutrition evidenced by features of decreased linearity, loss of subcutaneous fat, loose dry skin and sparse hairs (Lubchencar, et al, 1963; Naeye, 1966; Drillen, 1970 and Usher, 1970).

3. All the low birth weight babies (Premature and S.F.D.) were product of normal vaginal delivery and none of the newborns had any evidence of infection.

In both the group of cases blood sample was obtained from the umblical cord at the time of birth. Those samples not fulfilling the criteria of selection of low birth weight were discarded.

SELECTION OF CASES OF NEONATAL HYPERBILIRUBINEMIA :

Only those newborns were included in the study who were having a serum bilirubin level of 10 mg per 100 ml

Twenty full term normal weight newborns having plasma bilirubin levels ranging from 10.8 to 30 mg per 100 ml were included in the present study. Samples were taken from femoral vein in all the cases within a period of one week after birth. Care was taken to exclude those cases of neonatal hyperbilirubin with deep infection. Out of these 20 neonates having hyperbilirubinemia, 14 were having prolongation of Jaundice due to umblical sepsis, 4 had Rh incompatibility while the remaining 2 had physiological jaundice.

SELECTION OF CASES OF NEONATAL INFECTION :

Twenty newborns suffering from various infections were taken into consideration for this study. Six were having umblical sepsis, another six were having pyogenic meningitis, four had pneumonitis and remaining four had neonatal septicemia with multiple pyemic abscesses. The criteria for the selection of these cases was failure to suck, hypo or hyperthermia, episodes of cyanosis, convulsions and other systemic manifestations. Samples were obtained in all the cases from femoral vein within a period of one week.

Antenatal and Natal history -

A complete antenatal history pertaining to drug intake, irradiation, infections and systemic disease in the mother was taken into account. Natal history with

regard to rupture of membranes $\leq$ 12 hours or $\geq$ 12 hours, and mode of delivery was recorded in each case. The immediate post-natal history pertaining to apgar score, cry after birth and colour was noted in each case.

Examination of Newborn -

In all the newborns a detailed examination was done to detect any systemic disease. Examination of the baby was done in great detail with special reference to appearance, colour, cry, activity, cyanosis, jaundice, anaemia, any congenital malformation in the baby. Detailed examination was done to find out any source of infection in the form of septic focus or umblical sepsis or any other infection. Posture, reflexes, sucking was also noted in each case. Due emphasis was given to note the anthropometric measurements i.e. weight, length, chest and head circumference in each case. The gestational age was assessed in each case by the physical criteria of assessment as well as by the date of last menstrual period.

Investigation -

Diagnosis of type of neonatal hyperbilirubinemia was done by finding the level of total, direct and indirect bilirubin in the serum.

Cases of neonatal infection were confirmed by doing the total leucocyte count and differential leucocyte

count. X-ray chest and culture from local septic focus was done whenever needed. Diagnosis was confirmed by blood culture wherever needed.

Necessary investigations viz. blood counts, G.B.P., serum bilirubin, reticulocyte count, Coombs test, blood culture, blood grouping, X-ray chest, C.S.F. examination and smear (gram staining) were carried out wherever needed.

Collection of sample :

Blood samples for the present study were taken either from the umblical cord (in controls and low birth weights) at the time of delivery or from the femoral vein (in hyperbilirubinemia group and infection group). 5 ml sample of blood was collected from each case in a plain vial and serum was separated and was stored at -20°C for determination of immunological profile.

METHODS : Following immunological investigations were done in each case.

1. Serum immunoglobulin IgG & IgM.

2. Complement C_3 levels.

Immunoglobulin and complement determinations were done by the method of single radial diffusion of Mancini et al (1965). Solugen (R) S.R.I.D., Ready to use immuno-plates of each immunoglobulin supplied by M/S Immuno-

diagnostics Pvt. Ltd. were utilized. The blood samples taken from the cord at the time of birth or from femoral vein were centrifuged, sera was separated immediately by kept in deep freeze at -20°C, till the time of immunoglobulin determination.

Procedure :

Each immunoglobulin plate has 12 wells in which the different dilutions of standard reference sera and the serum samples were filled with the help of a capillary tube and care was taken not to underfill or over fill the wells. For accurate determination of IgG the patients serum was diluted five times with isotonic saline (1 part serum : 4 part saline), while no dilution was however done for estimation of IgM and complement C_3. After the wells were filled, the lid of the plate was replaced and the plate was left for the development of the precipitin ring in inverted position for 24 hours at room temperature. In case of estimation of IgM, plates were incubated at 4°C for another 24 hours. The ring diameters were measured by an immunomeasure and standard graph for each immunoglobulin were constructed using the values of the "Reference standard". The diameter (d^2) were plotted on the (↑) ordinate while the quantitative value (Reference standard values) were plotted on the (→) abscissa of the graph. Thereafter the values of the unknown samples were found out directly by interpolation and extrapolation on the standard graph. Results were expressed in mg/100 ml.

OBSERVATIONS

OBSERVATIONS

The present study "Immunological profile in newborn babies" was carried out in 80 neonates delivered or admitted in M.L.B. Medical College and allied Hospital, Jhansi, over a period of one year.

The cases selected for the present study were taken from various groups shown in Table 1.

Table - 1

Showing various study groups.

Sl. No.	Clinical groups	No. of cases
1.	Full term normal newborns (Control)	20
2.	Low birth weight infants	20
3.	Neonatal hyperbilirubinemia	20
4.	Neonatal infections	20
	Total	80

Table 2 shows the distribution of various study groups according to sex, gestational age and birth weight. It is evident from the table that male neonates predominated in our study (52) as compared to the females who numbered only 28. While the control group as well as cases of neonatal infection had equal number of male and female babies, males, far out-numbered the females in low birth weight group and in cases of neonatal hyperbilirubinemia. As mentioned earlier, barring the low birth weight babies, all three other groups viz. control, cases of hyperbilirubinemia and cases of neonatal infection were having normal gestational period and all were having weight more than 2.5 kg. as illustrated in table 2. However, the mean gestational age and birth weight of the low birth weight babies was 36.30 ± 3.65 and 1.62 ± 0.45 as depicted in the table 2. While the premature babies had gestational age and birth weight of 33.20 ± 2.28 and 1.30 ± 0.44, the gestational age and birth weight of S.F.D. babies was 39.40 ± 0.89 and 1.94 ± 0.11 respectively.

Table - 2

Showing distribution of various study groups according to sex, gestational age (G.A.) and birth weight.

Sl. No.	Study groups	No.of cases	Sex		Gestational age Mean ± S.D. (weeks)	Birth weight Mean ± S.D. (kg.)
			M	F		
1.	Control	20	10	10	39.38 ± 0.92	2.78 ± 0.13
2.	Low birth weight	20	16	4	36.30 ± 3.65	1.62 ± 0.45
	A. Premature	10	8	2	33.20 ± 2.28	1.30 ± 0.44
	B. I.U.G.R.	10	8	2	39.40 ± 0.89	1.94 ± 0.11
3.	Neonatal hyper-bilirubinemia	20	16	4	39.10 ± 1.52	2.76 ± 0.23
4.	Neonatal Infections	20	10	10	39.00 ± 0.94	2.55 ± 0.14

The immunological profile in our study was done in each case by determining the values of IgG, IgM and complement C_3 in all the groups of cases. Table 3 demonstrates these values in low birth weight babies both premature and IUGR, in comparison to the values observed in control group of cases. It is evident from table 3, that

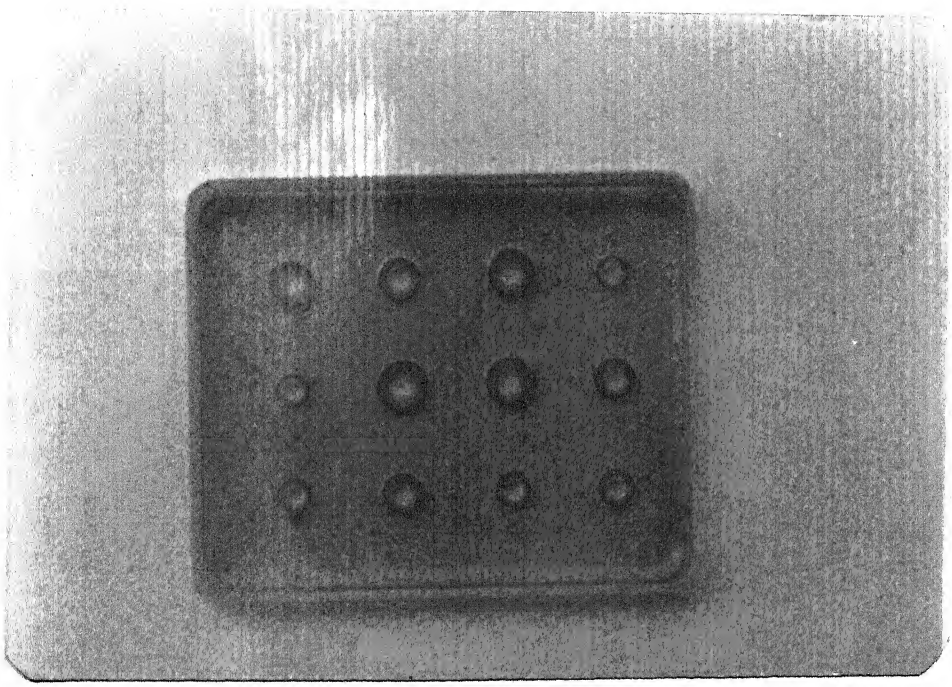
in the control group of cases the mean serum IgG and IgM values were 1365 ± 453.41 and 22.70 ± 4.76 mg% with a range of 1050 - 2500 and 17 to 28 mg% respectively. On the other hand it was seen, that low birth weight babies had lesser values of immunoglobulin IgG (1050.25 ± 515.00) with a range of 450 - 2000 mg% as compared to the control values, values being statistically significant ($P < 0.05$). Contrary to the IgG values, it is quite evident from the table that low birth weight babies had higher values of immunoglobulin IgM (30.05 ± 15.02 mg%) as compared to the values observed in the control group (22.7 ± 4.76 mg%) cases, there being a statistically significant difference between these two values as determined by student 't' test ($P < 0.05$). Low birth weight babies comprised of 10 cases each of premature and IUGR babies. Amongst the low birth weight babies, premature babies had much lower values of serum IgG (800.50 ± 232.38) as compared to the IUGR babies which had values of 1300.00 ± 501.24 mg%.

On comparison of the IgG values observed in low birth weight babies, it is evident that while there was a statistically significant difference ($P < 0.01$) in immunoglobulin IgG between the premature babies and the control group, no statistically significant difference was observed between I.U.G.R. babies and the control group ($P > 0.05$). Similarly, while analysing the IgM values, it was observed that premature babies demonstrated least levels of immunoglobulin IgM (18.1 ± 7.30), while highest value of IgM was seen in IUGR babies (42.0 ± 9.88). A comparison of IgM values observed in low birth weight babies revealed a statistically significant difference in both premature ($P < 0.05$) and IUGR babies ($P < 0.01$) as compared to the control group of cases. Further, on statistical analysis of IgG and IgM values in the sub-groups of low birth weight babies (premature and IUGR) it was observed that IUGR babies demonstrated higher values of both these immunoglobulins as compared to the premature babies ($P_G < 0.05$ and $P_M < 0.01$).

Table - 3

Showing serum IgG and IgM levels in control and low birth weight neonates.

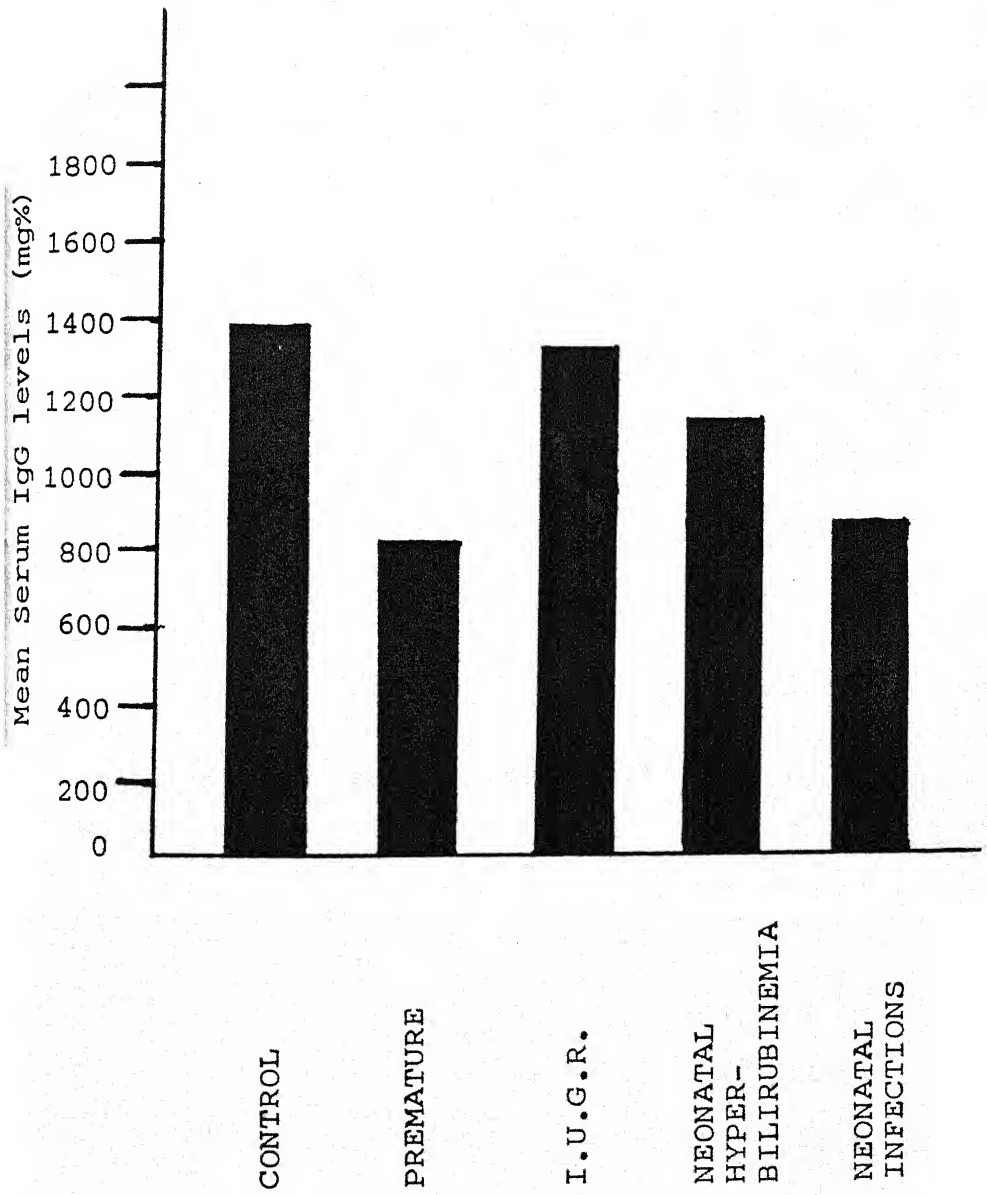
Sl. No.	Study group	No. of cases	Serum IgG levels Mean ±S.D. (mg%) Range (mg%)	Serum IgM levels Mean±S.D. (mg%) Range (mg%)	P_G	P_M
1.	Control	20	1365.00 ±453.41 (1050.00-2500.00)	22.70 ±4.76 (17.00-28.00)		
2.	Low birth weight neonates	20	1050.25 ±515.00 (450.00-2000.00)	30.05 ±15.02 (11.00-49.50)	< 0.05	< 0.05
	A. Premature	10	800.50 ±232.38 (450.00-1002.00)	18.1 ± 7.30 (11.00-28.00)	< 0.01	< 0.05
	B. I.U.G.R.	10	1300.00 ±501.24 (700.00-2000.00)	42.00 ±7.30 (25.00-49.50	> 0.05	< 0.01
Premature vs. I.U.G.R.			$P_G < 0.05$			
			$P_M < 0.01$			



PHOTOGRAPH SHOWING PRECIPITIN RINGS OF

IMMUNOGLOBULIN IgG.

MEAN SERUM IgG LEVELS IN CONTROL, PREMATURES, I.U.G.R., NEONATAL HYPERBILIRUBINEMIA AND NEONATAL INFECTIONS.



The immunological profile of cases of neonatal hyperbilirubinemia and its comparison with the values of immunoglobulins observed in control group of cases has been depicted in Table 4. Out of 20 cases having hyperbilirubinemia, 6 were having umblical sepsis, 4 had Rh incompatibility while the remaining 10 had physiological jaundice. It is evident from table 4 that neonates having hyperbilirubinemia, had lesser mean values of immunoglobulin IgG (1139.25 $\pm$ 319.85) with a range of 750 to 1550 mg%, as compared to the control values, though these values were not found to be statistically significant ($P > 0.05$). In contrast to the IgG values, it is evident from the table that neonates having hyperbilirubinemia had higher values of immunoglobulin IgM (50.60 $\pm$ 14.30 mg%) as compared to the values observed in the control group (22.70 $\pm$ 4.76 mg%) of cases, there being a statistically highly significant difference between these two values ($P < 0.01$).

Table - 4

Showing distribution of serum IgG and IgM levels in control and neonates having hyperbilirubinemia.

Sl. No.	Study group	No.of cases	Serum IgG levels Mean±S.D. (mg%) Range (mg%)	Serum IgM levels Mean±S.D. (mg%) Range (mg%)	P_G	P_M
1.	Control	20	1365.00 ±453.41 (1050.00-2500.00)	22.70 ±4.76 (17.00-28.00)		
2.	Neonatal byperbili-rubinemia	20	1139.25 ±319.85 (750.00-1550.00)	50.60 ±14.30 (27.50-65.00)	$\nearrow 0.05$	$\swarrow 0.01$

Table 5 shows the serum IgG and IgM values in neonates having infections and these values have been compared to the values observed in control group of cases. It is evident from this table that cases of neonatal infections had low values of IgG (865.00 ± 97.32) as compared to the values observed in control group of cases (1365.00 ± 453.41), values being highly statistically significant ($P_G \swarrow 0.01$) from each other. On the other hand a striking feature, as expected, was a much higher level of immunoglobulin IgM in cases of neonatal infection (66.05 ± 13.36 mg%) as compared to (22.70 ± 4.76 mg%)

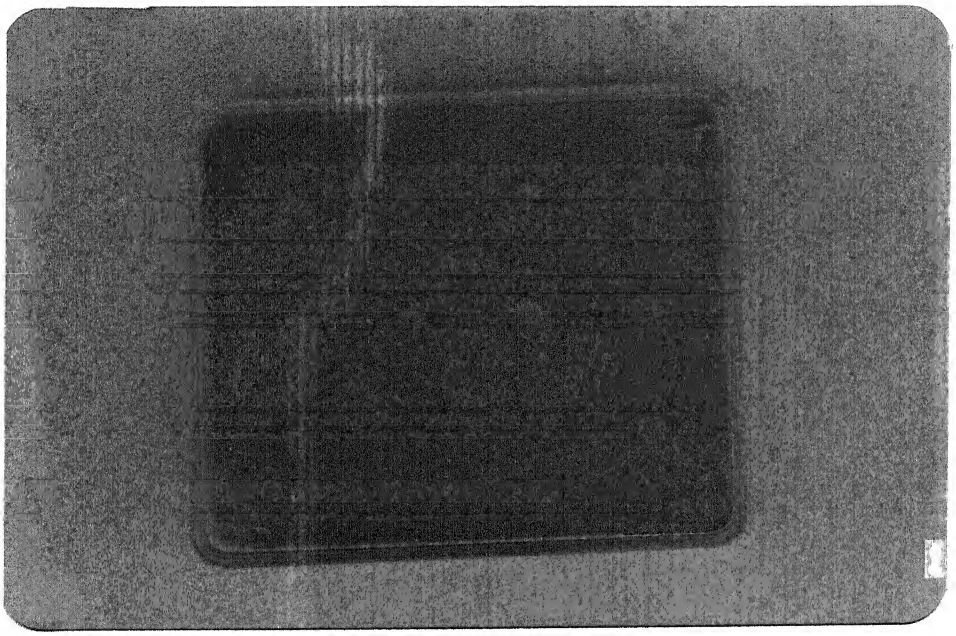
observed in control group of cases. These values, too, were found to be highly statistically significant ($P_M < 0.01$).

Table - 5

Showing distribution of serum IgG and IgM levels in control and neonates having infections.

Sl. No.	Study group	No.of cases	Serum IgG levels Mean±S.D. (mg%) Range (mg%)	Serum IgM levels Mean±S.D. (mg%) Range (mg%)	P_G	P_M
1.	Control	20	1365.00 ±453.41 (1050.00-2500.00)	22.70 ±4.76 (17.00-28.00)		
2.	Neonatal infections	20	865.00 ±97.32 (700.50-1050.00)	66.05 ±18.36 (27.50-85.00)	< 0.01	< 0.01

An effort was made to assess serum complement C_3 activity in the control as well as all our study group cases (Table - 6). It is evident from the table that in the control group of cases the mean serum complement C_3 value was 51.40 ± 18.76 mg% with a range of 34.50 to 74.50 mg%, while in low birth weight babies, the mean serum complement C_3 level was 42.55 ± 6.59 mg% with a



PHOTOGRAPH SHOWING PRECIPITIN RINGS OF

IMMUNOGLOBULIN IgM.

MEAN SERUM IgM LEVELS IN CONTROL, PREMATURE, I.U.G.R., NEONATAL HYPERBILIRUBINEMIA AND NEONATAL INFECTIONS.

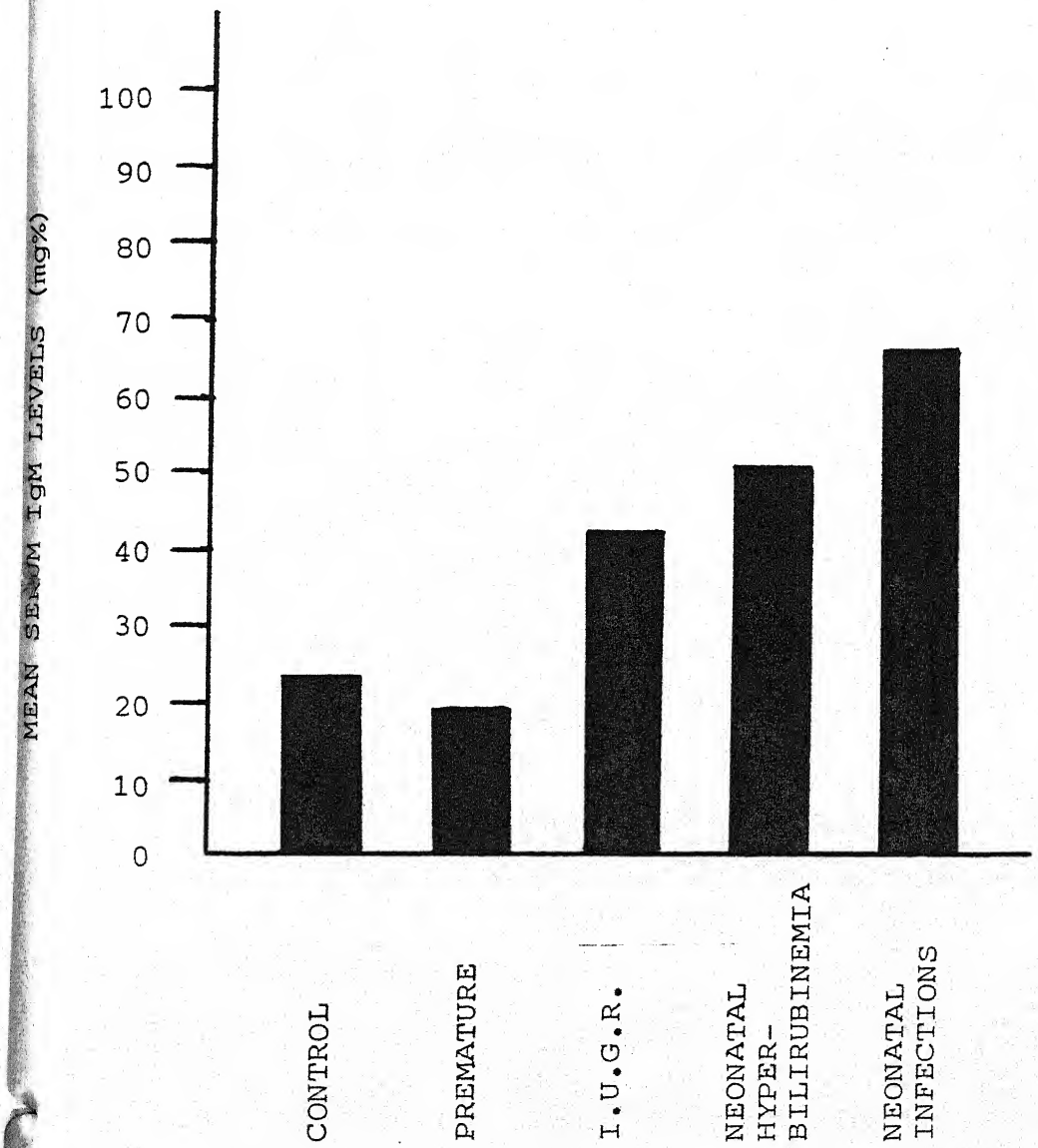


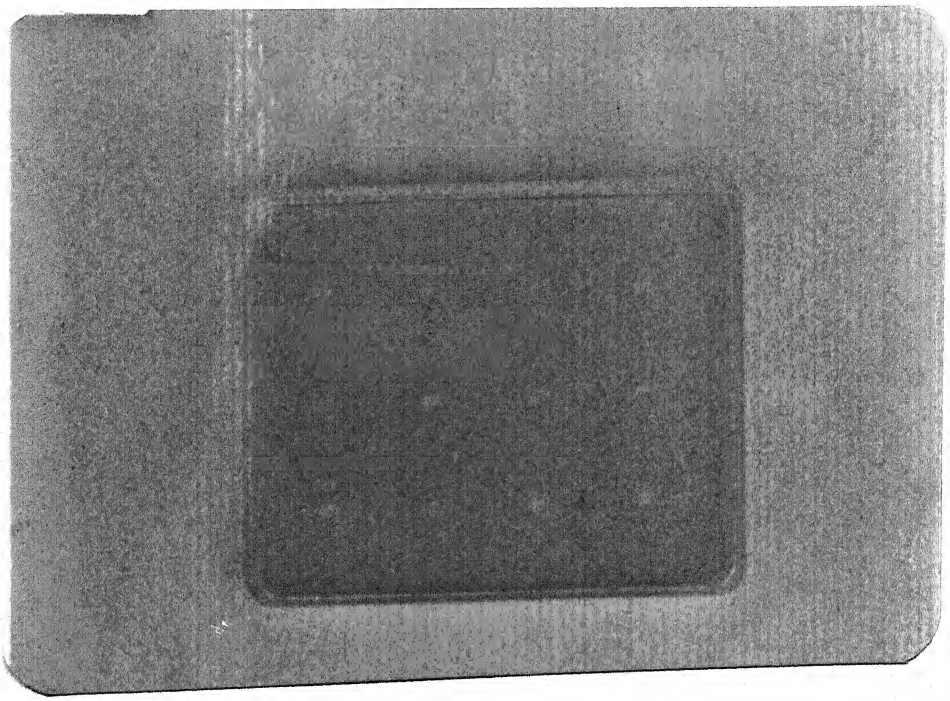
FIGURE - 2

When mean serum complement C_3 values were compared between the control and the low birth weight group, no statistically significant difference ($P_C > 0.05$) was observed. Amongst the low birth weight babies Table 6 reveals that premature babies had lower values of serum complement C_3 (38.90 $\pm$ 4.72 mg%) as compared to the I.U.G.R. babies which had values of 46.20 $\pm$ 6.49 mg%. On comparing the complement C_3 values observed in low birth weight babies it is evident that while there was a statistically significant difference ($P_C < 0.05$) in C_3 values between the premature babies and the control group, however no statistically significant difference was observed between IUGR babies and the control group ($P_C > 0.05$).

On further statistical analysis of C_3 values in the subgroups of low birth weight babies (premature and IUGR) it was observed that IUGR babies demonstrated higher values of complement C_3 as compared to the premature babies ($P < 0.05$).

It is also apparent from the table 6 that infants with hyperbilirubinemia were having mean serum complement C_3 value of 70.45 $\pm$ 23.65 mg% with a range of 44.00 to 110.50 mg% in contrast to the C_3 value (51.40 $\pm$ 18.76 mg%) with a range of 34.50 to 74.50 mg% obtained in the control group of cases. A comparison of these values to the values observed in the control group of cases revealed a highly significant rise ($P < 0.01$) of the serum complement C_3 level in the neonates having hyperbilirubinemia.

The values of serum complement C_3, as depicted in table 6, in neonatal infections group were in the range of 27.00 to 44.50 mg%, with the mean of 34.60 $\pm$ 6.87 mg% which were found to be markedly reduced in comparison with the C_3 values (51.40 $\pm$ 18.76 mg%) obtained in the control group. A comparison of these values with the control group revealed that serum complement C_3 levels were significantly higher ($P < 0.01$) in neonates with infections.



PHOTOGRAPH SHOWING PRECIPITIN RINGS OF COMPLEMENT C_3

MEAN SERUM COMPLEMENT C_3 LEVELS IN CONTROL, PREMATURE, I.U.G.R., NEONATAL HYPERBILIRUBINEMIA AND NEONATAL INFECTIONS.

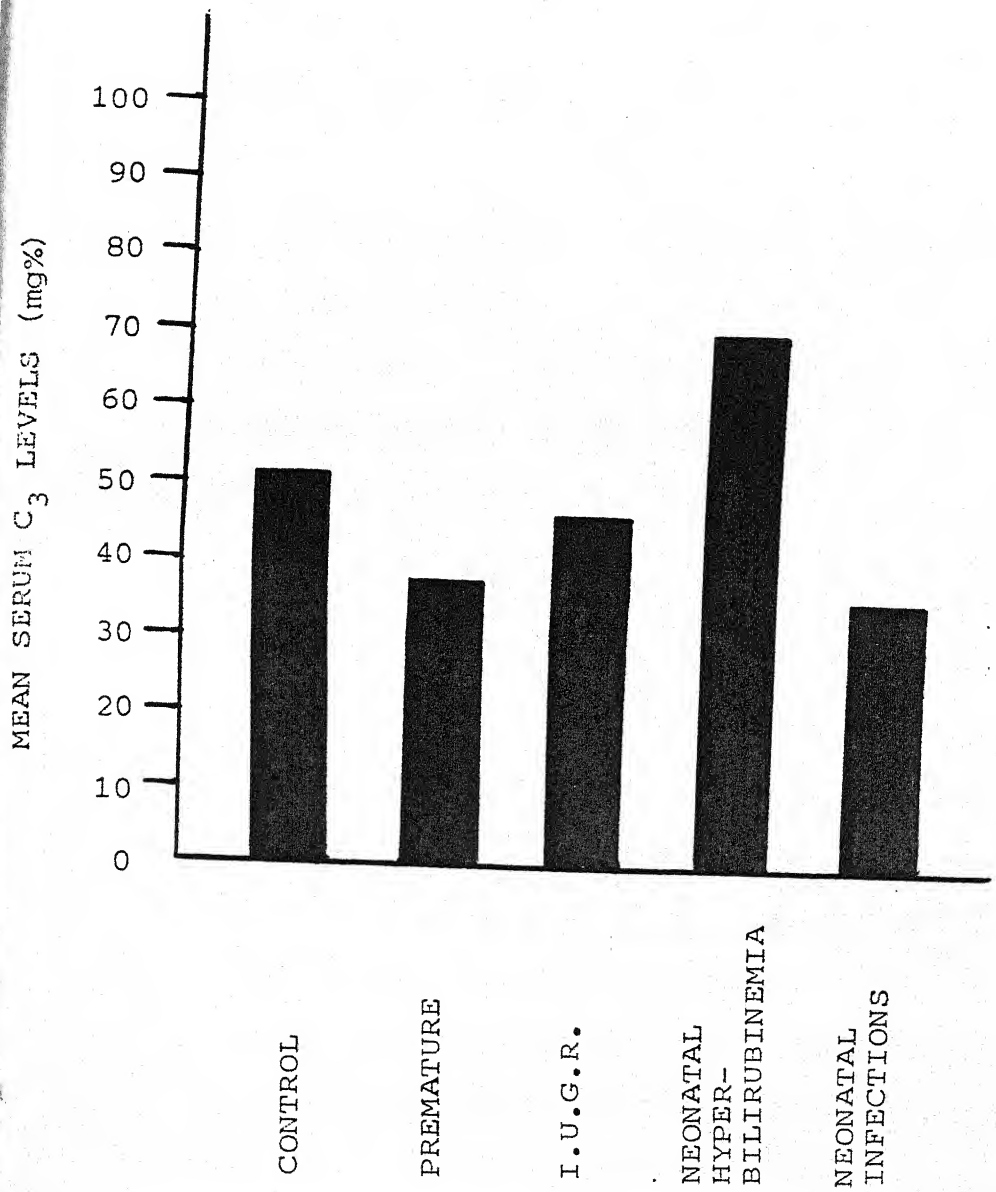


FIGURE - 3

An attempt was also made in our study to observe a correlation between different serum bilirubin levels to the changes in the Immunoglobulin and the complement C_3 activity in cases of neonatal hyperbilirubinemia. Accordingly cases of neonatal hyperbilirubinemia were sub-grouped into three categories depending on the serum bilirubin levels (Table 7).

Out of total 20 neonates having bilirubin levels more than 10 mg%, 8 were having bilirubin levels in betwee 10 to 15 mg%, 6 were having bilirubin levels in the range of 16 to 20 mg% while remaining 6 neonates had bilirubin levels of more than 20 mg%.

A significant finding of our study was that there was an inverse correlation between the increasing serum bilirubin levels to the decrease in the serum IgG as well as complement C_3 activity in cases of neonatal hyperbilirubinemia, values being maximally decreased (991.66 ± 218.42) in cases having serum bilirubin above 20 mg%. On statistical analysis, however, no significant difference was observed in these sub-groups for both immunoglobulin IgG and complement C_3 values (P_G & P_C $\overline{7}0.05$). Contrary to these findings, a direct correlation was seen in serum IgM values with increasing severity of jaundice, values being highest (53.34 ± 15.68) in those cases having serum bilirubin more than 20 mg%.

Table - 7

Showing distribution of serum IgG, IgM and complement C_3 levels in cases of neonatal hyperbilirubinemia according to various groups of bilirubin level.

Sl. No.	Range of Bilirubin (mg%)	No.of cases	Serum IgG Mean ± S.D. (mg%)	Serum IgM Mean ± S.D. (mg%)	Complement C_3 Mean ± S.D. (mg%)
1.	10 - 15	8	1316.75 ±395.56	47.50 ±17.85	73.50 ±19.30
2.	16 - 20	6	1150.00 ±400.00	50.75 ±12.28	70.83 ±21.12
3.	7 20	6	991.66 ±218.42	53.34 ±15.68	66.16 ±38.39

Table - 8

Showing P values obtained while comparing different groups of hyperbilirubinemia as shown in Table 7.

Sl. No.	Hyperbilirubinemia groups	P_G	P_M	P_C
1.	10-15 vs 16-20	70.05	70.05	70.05
2.	10-15 vs 7 20	70.05	70.05	70.05
3.	16-20 vs 7 20	70.05	70.05	70.05

As with neonatal hyperbilirubinemia, cases of neonatal infections were further sub-grouped according to the type of infections. Accordingly, twenty neonates having various infections were selected for the present study. Out of these 20 neonates, 6 had umblical sepsis, another 6 had pyogenic meningitis, 4 were having pneumonitis while remaining other 4 had neonatal septicaemia with multiple pyomic abscesses. E.coli was found to be the causative organism in 8 cases, out of which 2 were the causative organism for umblical sepsis, while other 6 lead to pyogenic meningitis. Staphylococcus aureus was isolated from the 4 neonates of umblical sepsis and 4 neonates of neonatal septicemia with multiple pyemic abscesses. In the remaining 4 infants, no causative organism could be isolated (Table - 9).

Table - 9

Showing causative organism in neonatal infections.

Sl. No.	Study group	No.of cases	Causative organisms		
			E.coli	Staph. aureus	Others
1.	Neonates with umblical sepsis	6	2	4	-
2.	Pyogenic meningitis	6	6	-	-
3.	Pneumonitis	4	-	-	-
4.	Neonatal septicemia with multiple pyemic abscesses	4	-	4	-
	Total	20			

Table 10 depicts the serum IgG, IgM and complement C_3 activity in the various types of neonatal infections just described. It is clearly evident from this table that whereas serum IgG values decreased with increasing severity of neonatal infection, the values of IgM on the contrary increased with severe infection. It is seen that cases of mild neonatal infection in the form of umblical sepsis had higher IgG values (866.67 $\pm$ 175.59) when compared to the severest form of neonatal infection (Neonatal septicemia with multiple pyemic abscesses) which had lowest values of IgG (800.00 $\pm$ 70.71 mg%). Since the number of cases in the sub-groups was too small, no statistically significant difference was observed between these two varieties of infections. However, statistically significant difference was seen between the IgG values observed in cases of pyogenic meningitis when compared to pneumonitis (P_G $\angle$ 0.05) and pyogenic meningitis when compared to multiple pyemic abscesses (P $\angle$ 0.01).

Regarding the IgM values, it is clear from the table that cases of septicemia with pyemic abscesses had the greatest rise of immunoglobulin IgM as compared to the less severe infection viz. umblical sepsis, pyogenic meningitis and pneumonitis, values being statistically significant (P $\angle$ 0.05). However, no statistically significant difference was seen in the IgM values between cases of neonatal sepsis as well as the values obtained in pyogenic meningitis and pneumonitis.

No significant difference was observed in Table - 10 in cases of the mean serum complement C_3 values in different types of infections ($P_C > 0.05$).

Table - 10

Showing distribution of serum IgG, IgM, and complement C_3 in cases of neonatal infections according to the type of the infection.

Sl. No.	Type of infection	No. of cases	IgG (mg%) Mean±S.D.	IgM (mg%) Mean±S.D.	Mean C_3 (mg%) Mean ± S.D.
1.	Neonates with umblical sepsis	6	866.67 ±175.59	51.16 ±25.11	37.8 ± 8.32
2.	Pyogenic meningitis	6	916.67 ± 28.86	64.00 ±14.02	33.16 ± 6.42
3.	Pneumonitis	4	862.50 ± 17.67	67.00 ± 6.36	32.75 ± 8.13
4.	Neonatal septicemia with multiple pyemic abscesses	4	800.00 ± 70.71	85.50	33.75 ± 9.54

Table - 11

Showing P values obtained while comparing different types of infections as depicted in table-10.

Sl. No.	Types of infections	P_G	P_M	P_C
1.	Neonates with umblical sepsis vs Pyogenic meningitis	$\nearrow 0.05$	$\nearrow 0.05$	$\nearrow 0.05$
2.	Neonates with umblical sepsis vs Pneumonitis	$\nearrow 0.05$	$\nearrow 0.05$	$\nearrow 0.05$
3.	Neonates with umblical sepsis vs Neonatal septicemia with multiple pyemic abscesses	$\nearrow 0.05$	$\swarrow 0.05$	$\nearrow 0.05$
4.	Pyogenic meningitis vs Pneumonitis	$\swarrow 0.05$	$\nearrow 0.05$	$\nearrow 0.05$
5.	Pyogenic meningitis vs Neonatal septicaemia with multiple pyemic abscesses	$\swarrow 0.01$	$\swarrow 0.05$	$\nearrow 0.05$
6.	Pneumonitis vs Neonatal septicaemia with multiple pyemic abscesses	$\nearrow 0.05$	$\swarrow 0.01$	$\nearrow 0.05$

We also attempted to observe the immunoglobulin levels in relation to the type of bacteria and to see whether there was a change in the immunological response with different bacterial species. Since the main causative organism in our study were E.coli and Staph. aureus, we only took up these bacteria and studied the response to the immunological status.

It is evident from Table - 12 that mean serum IgG levels were decreased in case of infection with staphylococcus aureus (787.00 $\pm$ 74.80 mg%) as compared to the values obtained in case of infection with E.coli (950.00 $\pm$ 70.71 mg%), which was also found to be highly significant on statistical analysis ($P < 0.01$). Contrary to the previous finding, mean serum IgM value was raised in cases of infection with staphylococcus aureus (74.25 $\pm$ 17.57 mg%) as compared to the mean value of serum IgM in cases of infection with E. coli (54.75 $\pm$ 21.58 mg%), but no statistical significance could be elicited ($P > 0.05$) on applying student 't' test. Table 12 and 13 also did not reveal any significant difference in the mean serum complement C_3 values obtained in the neonates infected either with E.coli or staphylococcus aureus ($P > 0.05$).

Table - 12

Showing distribution of serum IgG, IgM and complement C_3 values in cases of neonatal infections according to the causative organisms.

Sl. No.	Causative organism	No.of cases	IgG(mg%) Mean±S.D.	IgM(mg%) Mean±S.D.	Complement C_3 Mean ± S.D.
1.	E.coli	8	950.00 ±70.71	54.75 ±21.58	36.00 ±7.72
2.	Staphylo-coccus aureus	8	787.00 ±74.80	74.25 ±17.57	34.12 ±7.38

Table - 13

Showing P values obtained while comparing different types of infections according to the causative organism as shown in Table - 12.

Causative organisms	P_G	P_M	P_C
E. coli vs Staphylococcus aureus	< 0.01	> 0.05	> 0.05

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DISCUSSION

DISCUSSION

The present study has been carried out to study the immunological profile in 80 newborn babies, delivered in M.L.B. Medical College, Jhansi, over a period of one year. Our study group comprised of 20 cases each of low birth weight newborns, neonates with hyperbilirubinemia and those suffering from neonatal infection. An equal number of full term healthy normal weight healthy newborns delivered vaginally acted as control for the present study. The primary aim of our study was to evaluate the humoral immunity in all the study groups and compare these values with those observed in control group of cases.

An attempt was also made to assess the complement activity by measuring the C_3 levels in all the groups of cases which hitherto has not been attempted by many workers in the recent past. Besides evaluating the humoral immunity and complement activity, a thorough physical examination was done in each and every case to categorise the newborns in our study groups. Based on the observations depicted in table 1 to 13, various inferences has been drawn and discussed in details herewith.

Of the total 80 newborn babies selected for the present study, there were 52 male, while the rest 28 were female. As is evident from table 2, males predominated over the females in the low birth weight, as well as the group of cases of neonatal hyperbilirubinemia. Since the weight of the baby has a direct impact on the immunological status, care was taken to select only full term normal weight babies in all the groups except the group of low birth weight babies. The gestational age in our study was assessed by the morphological and neurological characteristics and tallied with the history of last menstrual period as given by mother. Immunoglobulin estimation was done by the method of single radial immunodiffusion technique of Mancini et al (1965).

Immunological profile in Control group of cases :

Serum IgG levels in the control group of cases had mean value of 1365 $\pm$ 453.41 mg% with range of 1050 - 2500 mg% while the serum IgM values had a mean of 22.70 $\pm$ 4.76 mg% with a range of 17 - 28 mg%.

Amongst the 20 control group of cases there were 8 cases having gestational age of 38 weeks, 2 cases of 39 weeks whereas 10 cases were of 40 weeks. On evaluating the IgG levels in these sub-groups of control group of cases a significant finding observed was that cases having gestational age of 40 weeks had mean values of 1610.00 $\pm$

549.31 mg% which was found to be highly significant from the mean values (1137.5 ± 110.86 mg%) observed in the 8 cases having gestational age of 38 weeks ($P < 0.05$). Our observation of the IgG levels therefore amply demonstrate that there is a linear correlation between increasing gestational age and the increasing levels of Immunoglobulin IgG. However, no such linear correlation of the increasing gestational age to the IgM values was observed in our study.

The immunoglobulin IgG values have been determined by various workers in the past two or three decades. Our values of 1365 ± 453 mg% in control group of cases are in conformity and more or less similar to the values observed by many other workers in the field (Hobbs and Davis, 1967, 1220 mg%, Evans et al, 1967, 1088 mg%, Sethi et al, 1980 1162.58 ± 137.43 mg%, Hariharan et al, 1984, 1259 ± 51 mg% and Kolhatkar et al, 1987 - 1377 mg%). However, few workers have reported much lower values in control group of cases viz. Yeung et al, 1968 (879 mg%), Prasad et al, 1971 (446.6 ± 20.4 mg%), Malik et al, 1977 (253.6 ± 137.5 mg%).

Kaur et al (1979) and Sharma et al (1986) are some of the workers to have reported higher values (1478.5 ± 305.71 mg% and 1402 ± 132.3 mg% respectively).

The exceptionally low levels of IgG in full term healthy control babies observed by Prasad et al (1971) and Kaur et al (1979) has been attributed by these workers to a

concomitently lower levels of IgG in the mothers of these babies. The view, by many of these workers (Hobbs & Davis, 1967; Yeung et al, 1968; Evans et al, 1971; Sethi et al, 1980; Tandon et al, 1984 and Bhatia et al, 1987), that there is a direct correlation of increasing IgG to the gestational age and birth weight has been substantiated in our study as has been discussed earlier.

The pattern of immunoglobulin IgM in full term neonates as observed by various workers has varied markedly in different series. As with IgG, only few workers viz. Hardy et al (1969) and Prasad et al (1971) have reported more or less similar IgM values of 20 and 17.88 mg% respectively. Most of the workers (Steihm & Fundenberg, 1966; Malik et al, 1977; Kaur et al, 1979; Khatua et al, 1984 and Sharma et al, 1986) have reported much lower values than that observed by us. The difference in the IgM values as observed by various workers may be attributed to the difference in the type of sample studied as well as the method of detection. No linear correlation of IgM values to the increasing gestational age and birth weight was observed by us or by any other worker in the field.

Immunological profile in low birth weight babies -

Twenty low birth weight babies which included 10 premature and 10 full term small for date babies were taken up for immunological assessment. Irrespective of the

sub-groups, the mean IgG and IgM values were 1050.25 ± 515.00 and 30.05 ± 15.02 mg% in our study, respectively. It was seen, that two cases weighing less than 1000 gms and having a gestational age of 30 weeks had the minimum values of immunoglobulin IgG (450 mg%) and IgM (11 mg%). On comparison of the IgG and IgM values observed in low birth weight babies to that of our control group of cases, it was seen that the IgG values were lower while the IgM values were higher, values in both being statistically significant ($P_G < 0.05$ and $P_M < 0.05$).

The decrease in immunoglobulin IgG in low birth weight babies in our study is mainly accounted by the premature babies in the low birth weight group, while the increase in the immunoglobulin IgM in low birth weight group babies when compared to the control is mainly accounted by rise of immunoglobulin IgM in IUGR group of babies. Amongst the low birth weight babies, it was observed (Table 3) that premature babies had mean values of IgG and IgM 800.50 ± 232.38 and 18.1 ± 7.30 mg% while IUGR babies had mean valves of IgG and IgM 1300.00 ± 501.24 mg% and 42.00 ± 9.88 mg% respectively. As has already been mentioned, the two most premature babies of 30 weeks gestational age having birth weight of 800 gm each, manifested the least values of serum IgG. It is evident from our observation that the premature babies manifested a highly significant decrease (800 mg%) as compared to the control values (1365.00 ±

453.41 mg%), values were found to be highly significant ($P < 0.01$). However, no statistically significant difference was observed in the IgG values in the IUGR babies and the control group ($P_G > 0.05$). Since the major portion of the IgG of the newborn is derived transplacently from the mother continuously during the third trimester of pregnancy, the decrease in level of this class of immunoglobulins in premature babies can be accounted for the shorter period of gestation available in these neonates for the transfer of this immunoglobulin.

The IUGR babies did not show any significant difference in the IgG levels from the control group of cases, possibly due to the fact that all our IUGR babies were having mild intra-uterine growth retardation. Similarly on analysing the IgM values it was observed that premature babies demonstrated lower levels of immunoglobulin IgM while IUGR babies manifested with highest level of IgM, both these values were found to be statistically significant from that of control group of cases. The rise of IgM in IUGR babies is easily explainable since all these babies were an outcome of deliveries in which the mother had some systemic disease or infection.

Umpteen workers in the past have evaluated the humoral immunity in the low birth weight babies and practically all of them are of the opinion that premature babies manifest with decreased level of IgG, while the IUGR

Various workers have studied the levels of immunoglobulin IgG and IgM in premature and small for date infants separately. In our study, value of immunoglobulin IgG in premature babies (800.5 mg%) was found to be more or less similar to the values obtained by other workers in the field (Evans et al, 1971 - 850 mg%; Singh et al, 1978 - 691.92 ± 242.4; Kaur et al, 1979 - 829.66 ± 174.09 and Tandon et al, 1984 - 708 ± 195.3 mg%). Few workers have reported much lower values in premature babies viz. Yeung & Hobbs, 1968 - 512 mg%; Prasad et al, 1971 - 467 ± 85.16 mg% and Sethi et al, 1978 - 478.2 ± 70.71 mg% ; Raghvan et al, 1976 - 1366.6 ± 792.8 mg%; Shapiro et al, 1981 - 1363 ± 357 mg% and Sharma et al, 1986 - 1267.3 ± 84.0 mg% are the few workers to have reported higher values.

The decrease in the IgG levels in premature babies, in our study as well as the values reported by others, (as has already been suggested) is due to the decreased transplacental passage of immunoglobulin IgG in the last trimester of pregnancy. The workers who have reported higher values of IgG in premature babies than us too, had significantly low levels of IgG as compared to their control, which had nearly double the values (2028.8 ± 1100.0 mg%).

A significant finding in our study was that each and every premature baby had measurable immunoglobulin IgM, which is in contrast to some other workers who have

not reported measurable levels of IgM in all the premature babies (Kaur et al, 1979; Tandon et al, 1984 and Sharma et al, 1986).

Our observations of low IgM values in premature babies as compared to our controls has been substantiated by other workers too, viz. Prasad et al, 1976 - 6.6 ± 3.6 mg%, Raghvan et al, 1976 - 10.04 ± 7.51 mg%, Singh et al 1978 - 12.5 ± 12.32 mg%, Sharma et al, 1986 - 7.4 ± 3.9 mg%. This observation goes to prove that immunoglobulin IgM has got no correlation to the gestational age as has also been mentioned by these workers.

Amongst the IUGR babies as has been mentioned earlier, no significant difference was observed in the IgG values as compared to our control group of cases. Our values of 1300.00 ± 501.24 mg% in IUGR babies are in conformity to those of some workers (Singh et al, 1978; Kaur et al, 1979; Shapiro et al, 1981; Tandon et al, 1984 and Sharma et al, 1986), while few workers have reported low values than us viz. Sethi et al, 1980 and Khatua et al, 1984.

Although in our study we did not categorize cases of IUGR into mild ($\angle$ 10th percentile), moderate ($\angle$ 3 to 10th percentile) and severe ($\angle$ 3rd percentile) IUGR groups, Kaur et al (1979) and Tandon et al (1984) having done this, reported significantly low values of IgG from the controls only in the severe IUGR group of cases. The mean

level of IgM obtained in our study in IUGR babies was 42.00 ± 9.88 mg% which is significantly higher than the value obtained in control (22.70 ± 4.76 mg%) group of cases ($P < 0.01$). Value observed by us, in our series is much higher as compared to the values given by other workers, viz. Singh et al (1978) - 13.6 ± 6.55 mg%, Kaur et al 1.76 ± 4.7 mg% and Khatua et al (1984) - 12.78 ± 24.36 mg%. The difference in the IgM values as observed by various workers can be due to the type of sample studied as well as the method used for the assessment of the immunoglobulin. Higher mean values of IgM in IUGR babies in our study was possibly due to intra-uterine infection in these cases which triggered and heightened the levels of IgM in these cases.

Immunological profile in cases of Neonatal Hyperbilirubinemia -

Twenty neonates having bilirubin level between 10.8 mg% and 30 mg% were selected for the present study. The mean IgG and IgM values were 1339.25 ± 319.85 mg% and 50.60 ± 14.30 mg% respectively in our study.

It is evident from our observations (Table 4) that infants with hyperbilirubinemia did not reveal any significant alteration in the mean serum IgG values, as compared to control group of cases ($P_G > 0.05$).

Contrary to the mean IgG values, the mean value of IgM was higher (50.60 ± 14.30) in the neonatal hyper-

bilirubinemia group as compared to the control group of cases (22.70 ± 4.76 mg%) values being statistically significant (P_M < 0.01).

Very few workers have assessed the humoral immunity in cases of neonatal hyperbilirubinemia. However, Ansaldi et al (1968) found a slight decrease in the levels of IgM levels in neonates with serum bilirubin levels above 16 mg%. However, Mantalenaki et al (1975) did not find any significant difference in the levels of immunoglobulins IgG and IgM between hyperbilirubinemic infants and controls. Sethi et al (1989) have been the only worker in the recent past to have recorded values of immunoglobulin IgG in cases of neonatal hyperbilirubinemia. Their values of 995.87 ± 163 mg% were more or less similar to the IgG values in our study. The rise of IgM in cases of neonatal hyperbilirubinemia could possibly be because of associated infections in many of our cases, which is substantiated by the fact that highest values of IgM were found in severe neonatal hyperbilirubinemia which are more prone to infection. Since there is paucity of data in the humoral immunity in cases of neonatal hyperbilirubinemia, no comparison could be done in this regard.

Thus in nutshell we see that hyperbilirubinemia per se has no effect on the immunoglobulin IgG, though the values of IgM may be increased due to associated infection. However, further work has to be done to substantiate our

Immunological profile in Neonatal Infections group -

Twenty newborn infants suffering from various infections were selected for the present study. Care was taken to select only full term normal infants to exclude the detrimental effects of prematurity or fetal malnutrition on the immunological apparatus of the studied cases. All the cases were subjected to various tests for the assessment of serum IgG and IgM in neonates having infections. The mean IgG and IgM values were 865.00 ± 97.32 mg% and 66.05 ± 18.36 mg% respectively in study conducted by us. It was seen, that, whereas the cases of neonatal infection had lower values of IgG as compared to the control ($P < 0.01$), values of immunoglobulin IgM were found to be much higher as compared to the control group of cases ($P < 0.01$). This significant finding of decrease in IgG and increase in serum IgM in cases of neonatal infection is easily explainable on the basis, that depression of IgG is the cause of neonatal infection whereas the rise of IgM is effect of infection.

The mean IgG value in our study (865.00 ± 97.32 mg%) was much higher as compared to the value obtained by Prasad et al (1971) - 479.8 ± 32.4 mg% and Malik et al 243.6 ± 107.3 mg%, though unlike us, these workers did not observe a decrease in IgG values as compared to the control group of cases.

The findings of raised levels of immunoglobulin IgM in response to infections have been uniformly observed by several other workers viz. Alford et al (1967) - 13 - 130 mg%, Hardy et al (1969) - 30 mg%, Khan et al (1969) - 55 mg%, Prasad et al (1971) - 39.2 ± 8.36 mg% and Malik et al (1977) - 98.7 ± 58.7 mg%. The elevated levels of immunoglobulin IgM as a response to infection is possibly an exaggeration of the normal response to a myriad of antigens in the extra-uterine environment.

Complement C_3 activity in various study groups -

Complement C_3 was assessed in all 80 newborn babies selected for the present study. As is evident from Table - 6, the mean C_3 value in control group of cases was 51.40 ± 18.76 mg%. Tandon et al (1984) have reported nearly similar values of complement C_3 (51.50 ± 14.94 mg%) as reported by us. However, Kaur et al (1979) and Shapiro et al (1981) have reported a higher mean value of complement C_3 in their control group of cases (124.72 ± 44.62, and 90 ± 18 mg%) respectively. In the low birth weight babies, the mean serum complement C_3 levels was 42.55 ± 6.59 mg% which was found to be statistically insignificant from control group values ($P > 0.05$). A significant finding in the complement activity of the low birth weight babies was, that premature babies had statistically significant lower values ($P < 0.05$) as compared to the controls, whereas the values of C_3 in IUGR

group had no statistical significance ($P > 0.05$) from the control group of cases. The depression of complement activity in premature babies is well documented fact in literature, which accounts for one of the factors enhancing infection in the premature babies because of depressed complement activity.

Very few workers have assessed complement C_3 in low birth weight babies. Tandon et al (1984) have reported nearly similar values in premature and IUGR babies as observed in our study (33.8 ± 11.18 mg% and 47.5 ± 19.75 mg%). However, Kaur et al (1979) have reported much higher values of complement C_3 in premature babies than our values, though like us their values in premature babies too were much low than the values in their control group of cases. Kaur et al (1979) like us, too have opined, that pre-term babies have lower levels of complement in proportion to their immaturity and since complement plays a role in the heat labile opsonic system and enhances phagocytosis of organisms, a depression of this factor of immune response predisposes the premature baby to greater infection. Another fact highlighted by our observation was, that IUGR babies had more or less normal complement activity, akin to that observed in full term babies.

Complement activity which was assessed in all the 20 cases of neonatal hyperbilirubinemia had mean value of 70.45 ± 23.65 mg% which was found to be higher and

statistically significant from the values observed in the control group of cases (P_C < 0.01). No study till date has been found with regards to the complement activity in cases of neonatal hyperbilirubinemia. The rise of complement activity in these cases is not easily explainable since some of the cases of neonatal hyperbilirubinemia were also having infection but Cooper et al (1967) have reported that complement activity may be normal or elevated earlier in the disease and declines in the late terminal stages of the infection. Why there was a rise of complement activity in hyperbilirubinemia remains still to be answered and further work has to be done to elucidate the effect of hyperbilirubinemia on the complement activity.

It is evident from Table - 6 that C_3 complement levels in the neonates having infections (34.60 ± 6.87 mg%) were significantly lower as compared to the non-infected control group of cases (P_C < 0.01). Few workers in the past have also reported low complement C_3 activity in the infected newborns (R.P. Singh, 1986).

A decrease of complement activity in infected newborns has been explained on the basis of consumption of various components of the complement system in various infections.

Immunological profile according to severity of neonatal hyperbilirubinemia -

In our study we also tried to find out a correlation between different serum bilirubin levels to the changes in the immunoglobulin and the complement C_3 activity in cases of neonatal hyperbilirubinemia. Accordingly, cases of neonatal hyperbilirubinemia were sub-grouped into three categories depending on the serum bilirubin levels (Table - 7).

A significant finding of our study was that there was an inverse correlation between the increasing serum bilirubin levels to the decrease in the serum IgG as well as complement C_3 activity in cases of neonatal hyperbilirubinemia, values being maximally decreased (991.66 ± 218.42) in cases with serum bilirubin above 20 mg%. However, on statistical analysis no significant difference was observed in the various sub-groups, as described in table - 7, for both immunoglobulin IgG and complement C_3 values (P_G & $P_C > 0.05$). On the other hand a direct correlation was observed in serum IgM values with increasing severity of jaundice, values being highest (53.34 ± 15.68 mg%) in those cases having serum bilirubin more than 20 mg%.

Besides our best efforts we could not compare our findings due to paucity of the data in this field.

Immunological profile of neonates having infection according to the severity and causative organism -

An attempt was also made in our study to observe a correlation of the severity of neonatal infection, to the immunological profile of the newborn babies. It is evident from our observations (Table - 10) that whereas serum IgG values decreased with increasing severity of neonatal infection, the values of IgM on the contrary increased with severe infection. Neonatal septicemia with multiple pyemic abscesses, the most severe form of neonatal infection in our study, recorded the lowest values of IgG and the highest values of immunoglobulin IgM. However, cases of umblical sepsis, the milder form of neonatal infection recorded higher values of immunoglobulin IgG and low levels of IgM. As has been explained earlier, a fall and rise of IgG and IgM respectively are the cause and effect of neonatal infection having a direct bearing on the severity of the neonatal infection.

Since none of the worker in the past have evaluated the IgG level in cases of neonatal infection, a comparison to our values could not be ascertained. Khan et al in 1969 have however reported high values of immunoglobulin IgM in 88% cases of meningitis and pneumonia.

Highlight of the present study in neonatal infection was, that staphylococcus aureus was found to have a greater

impact on the humoral status of the newborn baby as compared to the E. coli infection. It is evident from table - 12 that cases of staphylococcal infection had a more profound alteration of the humoral immune system as manifested by a significant decrease of immunoglobulin IgG when compared to an equal number of cases of E. coli infection ($P_G \angle 0.01$) and a greater rise of immunoglobulin IgM than that observed with E. coli infected cases. However, further work has to be done in greater number of cases to substantiate these findings.

Complement activity however was found to have no significant difference as regards the nature or the causative organism of the neonatal infection.

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SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The present work has been carried out in the Department of Pediatrics, M.L.B. Medical College and Allied Hospital, Jhansi, with active collaboration of Department of Obstetrics and Gynaecology. Eighty newborns belonging to various clinical groups were subjected to immunological studies for the purposes of the present study. Out of these, 20 were normal healthy full-term neonates taken as control, 20 were low birth weight infants, 20 cases were of neonatal hyperbilirubinemia, while the remaining 20 newborns had infections. All the cases were selected only after satisfying the selection criteria for each study group. A detailed history and physical examination of the mother was done along with special stress over the antenatal and natal factors. Necessary investigations to confirm the diagnosis, were carried out in each case. All the newborns were subjected to various immunological tests for assessment of humoral immunity according to the method given by Mancini et al (1965) and modified by Fahey et al (1965).

Brief account of the work conducted in the present study is being summarised here.

Group I (CONTROL) Full term normal newborns -

Twenty full term normal, healthy newborns acted as control in the present study. Following are the values of immunoglobulins and complement C_3 in the control group of cases.

Serum IgG levels

The mean serum IgG level in the cord blood of the control group of cases was 1365.00 ± 453.41 mg%, with a range of 1050.00 to 2500.00mg%.

Serum IgM levels

The values of serum IgM, obtained in control cases had a mean ± S.D. of 22.70 ± 4.76 mg%, with a range of 17.00 to 28.00 mg%.

Serum complement C_3 levels

The values of serum complement C_3 in the cord blood of the control group had a mean ± S.D. of 51.40 ± 18.76 mg% with a range of 34.50 to 74.50 mg%.

Amongst the 20 control group of cases, there were 8 cases having gestational age of 38 weeks, 2 cases of 39 weeks and 10 cases of 40 weeks. On evaluating the IgG levels in these sub-groups of control group of cases, a significant finding observed was, that cases having

gestational age of 40 weeks had mean values of 1610.00 ± 549.31 mg%, which was found to be highly significant from mean values (1137.5 ± 110.86 mg%) observed in the 8 cases having gestational age of 38 weeks ($P < 0.05$). Our observation of the IgG levels in normal healthy full term newborns, therefore amply demonstrate, that there is a linear correlation between increasing gestational age and the increasing levels of immunoglobulin IgG. However, no such correlation of the increasing gestational age to the IgM and complement C_3 values was seen in our study.

Group II - Low birth weight newborn babies -

Twenty low birth weight babies (less than 2500 gms.), which included both premature and IUGR (10 cases in each group) babies constituted this group of our study. Following are the values of IgG, IgM and complement C_3, obtained in our study.

Serum IgG levels

The mean value of immunoglobulin IgG in low birth weight infants was 1050.25 ± 515.00 mg% with a range of 450.00 to 2000.00 mg%. These values were found to be significantly lower when compared with the control group ($P_G < 0.05$).

The mean value of immunoglobulin IgG in premature babies was 800.50 ± 232.38 mg% with a range of 450.00 to 1002.00 mg% and this value was significantly lower ($P_G < 0.01$) as compared to the control group.

The value of IgG in the IUGR babies had a mean ± S.D. of 1300.00 ± 501.24 mg% with a range of 700.00 to 2000.00 mg%. However, a comparison of these values with the control group did not reveal any significant difference (P_G $\overline{>}$ 0.05).

On statistical analysis of IgG values in sub-groups of low birth weight babies, it was observed that IUGR babies demonstrated higher values as compared to the premature babies and the difference was statistically significant (P_G < 0.01).

The decrease observed in the IgG levels in low birth weight babies in our study is mainly accounted by the premature babies. This is further substantiated by the fact that two most premature babies of 30 weeks gestational age, having birth weight of 800 gm each, manifested the least values of serum IgG. Since the major portion of the IgG of the newborn is derived transplacently from the mother during the third trimester of pregnancy, the decrease in the level of this class of immunoglobulins in premature babies can be accounted by the shorter period of gestation available in these neonates for the transfer of this immunoglobin IgG.

The IUGR babies did not show any significant difference in the IgG levels from the control group of cases, possibly due to the fact, that all our IUGR babies were having mild intra-uterine growth retardation.

Serum IgM levels

The mean value of IgM in low birth weight neonates was 30.05 ± 15.02 mg% with a range of 11.00 to 49.50 mg%. On comparison of these values with that observed in the control group of cases, a significant increase was observed (P_M < 0.05) in the low birth weight group as compared to the control.

The mean value of immunoglobulin IgM in premature babies was 18.1 ± 7.30 mg% with a range of 11.00 to 28.00 mg% and this value was significantly lower (P_M < 0.05) as compared to the values observed in the control group of cases.

The value of IgM in the IUGR babies had a mean ± S.D. of 42.00 ± 9.88 mg% with a range of 25.00 to 49.50 mg%. On comparison of these values with the values observed in the control group, a significant increase (P < 0.01) was observed in the intra-uterine growth retarded babies.

When a comparison of the IgM values was done between the sub-groups of low birth weight babies, a significantly higher difference was observed in the values of IgM (P_M < 0.01) in the IUGR babies.

The increase observed in the serum IgM levels in low birth weight babies in our study is mainly accounted by rise of immunoglobulin IgM in IUGR group of babies.

It was also observed in our study, that premature babies demonstrated lower levels of immunoglobulin IgM while IUGR babies manifested with highest level of IgM. The rise of IgM in IUGR babies is easily explainable since all these babies were an outcome of deliveries in which the mother had some systemic disease or infection. The low levels of IgM in premature babies goes to prove that immunoglobulin IgM has got no correlation to the gestational age.

Serum complement C_3 levels

The mean serum complement C_3 value of the low birth weight babies was 42.55 $\pm$ 6.59 mg% with a range of 31.50 to 57.50 mg% which did not reveal any significant difference when compared to the control group value ($P > 0.05$).

The mean value of complement C_3 in the premature babies was 38.90 $\pm$ 4.72 mg% with a range of 31.50 to 44.50 mg%. These values were found to be significantly lower when compared to the values observed in the control group of cases ($P_C < 0.05$).

The mean serum complement C_3 value in the low birth weight IUGR babies was 46.20 $\pm$ 6.49 mg% with a range of 41.50 to 57.50 mg%. No significant difference was observed when this value was compared with that observed in the control group ($P_C > 0.05$).

When complement C_3 values obtained in the sub-groups of low birth weight babies were compared with one another, it was observed that IUGR babies had higher values as compared to the premature babies ($P_C \angle 0.05$).

A significant finding of complement C_3 activity in low birth weight babies was, that premature babies had lower values of serum complement C_3. The depression of complement activity in premature babies is well documented fact in literature, which accounts for one of the factors enhancing infection in the premature babies. Another fact highlighted by our study was that IUGR babies had more or less normal complement activity, akin to that observed in full term babies.

Group III - Neonatal hyperbilirubinemia -

Twenty neonates having neonatal hyperbilirubinemia of 3 to 6 days duration, bilirubin level ranging from 10.8 mg% to 30 mg%, with a mean of 19.34 $\pm$ 7.33 mg% constituted the present group in our study. Out of these 20 neonates, 14 were having prolongation of physiological jaundice due to umblical sepsis, 4 had Rh incompatibility and remaining 2 neonates had jaundice within physiological limits.

The values of immunoglobulins and complement C_3 detected in our study are as follows -

Serum IgG levels

The mean value of serum IgG in neonates having hyperbilirubinemia was 1139.25 ± 319.85 mg% with a range of 750 to 1550 mg per 100 ml. It is evident from our study, that infants with hyperbilirubinemia did not reveal any significant alteration in the mean serum IgG values as compared to control group of cases ($P_G > 0.05$). It is thus evident from our study that hyperbilirubinemia per se has no effect on the immunoglobulin IgG.

Serum IgM levels

The mean value of serum IgM in the neonatal hyperbilirubinemia group was 50.60 ± 14.30 mg% with a range of 27.50 to 65.00 mg per 100 ml. The mean value of IgM was higher in the neonatal hyperbilirubinemia group as compared to the control group of cases, values being statistically significant ($P_M < 0.01$).

The rise in the IgM level in cases of hyperbilirubinemia could possibly be because of associated infections in many of our cases, which is substantiated by the fact that highest values of IgM were found in severe neonatal hyperbilirubinemia which are more prone to infection.

Serum complement C_3 levels

Infants with hyperbilirubinemia were having mean serum complement C_3 values of 70.45 ± 23.65 mg% with a

range of 44.00 to 110.50 mg%. A comparison of these values to the values observed in the control group of cases revealed a highly significant rise ($P < 0.01$) of the serum complement C_3 level in the neonates having hyperbilirubinemia.

A rise in the values of complement C_3 activity was observed in our study in cases of neonatal hyperbilirubinemia. This rise of complement activity in these cases is not easily explainable since many of the cases of neonatal hyperbilirubinemia were also having umblical sepsis. Why there was a rise of complement activity in hyperbilirubinemia remains still to be answered and further work has to be done to elucidate the effect of hyperbilirubinemia on complement activity. However, it has been mentioned in literature that complement activity may be normal or elevated earlier in the disease and declines in the late terminal stages of the infection.

In our study, we also observed an inverse correlation between the increasing serum bilirubin levels to the decrease in the serum IgG as well as complement C_3 activity in cases of neonatal hyperbilirubinemia, values being maximally decreased in cases with serum bilirubin above 20 mg%. Contrary to this a direct correlation was observed in serum IgM values with increasing severity of jaundice, value being highest in those cases having serum bilirubin more than 20 mg%.

Group IV - Neonatal infections -

Twenty newborns suffering from various infections were selected for the present study. Out of these 20 neonates, 6 had umblical sepsis, another 6 had pyogenic meningitis, 4 were having pneumonitis while remaining other 4 had neonatal septicemia with multiple pyemic abscesses. All the cases having infections were subjected to various tests for the assessment of serum IgG, IgM and complement C_3. The values of immunoglobulins and complement C_3 observed in our study are as follows -

Serum IgG levels

The mean IgG value in cases of neonatal infections group was 865.00 $\pm$ 97.32 mg% with a range of 700.50 to 1050.00 mg%. These values were found to be significantly lower than compared to the values observed in the control group of cases (P_G $\angle$ 0.01).

This significant finding of decrease in IgG in cases of neonatal infection is easily explainable on the basis, that decreased levels of IgG is the cause of infection in the newborn babies.

Serum IgM levels

The mean value of immunoglobulin IgM in cases of neonatal infections group was 66.05 $\pm$ 18.36 mg% with a range of 27.50 to 85.00 mg% which was significantly higher

The significant findings of raised levels of immunoglobulin IgM in response to infections is possibly an exaggeration of the normal response to a myriad of antigens in the extra-uterine environment.

Serum complement C_3 levels

The mean value of complement C_3 in cases of neonates having infections, was 34.60 $\pm$ 6.87 mg% with a range of 27.00 to 44.50 mg%. A comparison of these values to the values observed in the control group of cases revealed a significant decrease of the serum complement C_3 level in the neonates having infections.

This significant finding of decreased complement C_3 activity in infected neonates could be explained on the basis of consumption of various components of complement system in various infections.

An attempt was also made in our study to observe a correlation of the severity of neonatal infection, to the immunological profile of the newborn babies. It is evident from our study, that whereas serum IgG levels decreased with increasing severity of neonatal infection, the values of IgM on the contrary increased with severe infection. Neonatal septicemia with multiple pyemic abscesses, the most severe form of neonatal infection in our study, recorded the lowest values of IgG and the highest values of immunoglobulin IgM. However, cases of umblical sepsis,

the milder form of neonatal infection recorded higher values of IgG and low levels of IgM. A fall and rise of IgG and IgM respectively are the cause and effect of neonatal infection.

Another highlight of the present study in neonatal infection was, that staphylococcus aureus was found to have a greater impact on the humoral immune status of the newborn baby as compared to the E. coli infection. However, further work is needed in larger number of infected babies to confirm the above observations.

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